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SPECIAL LISTING

CURRENT CANCER RESEARCH

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DEVELOPMENT AND APPLICATION OF IN VITRO TECHNIQUES

SEPTEMBER 1981

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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RESEARCH AREAS

	Abstract Number
I. TECHNIQUES IN THE CHARACTERIZATION OF SOMATIC CELL HYBRIDS	1
A. GENETIC ANALYSIS OF HYBRIDS BY SOMATIC MAPPING AND MUTATION	1
B. GENETIC ANALYSIS OF SOMATIC CELL HYBRIDS BY OTHER SPECIFIC TECHNIQUES	12
C. IDENTIFICATION OF HYBRID REGIONS IN SOMATIC CELLS BY IMMUNOLOGIC TECHNIQUES	25
D. OTHER SPECIFIC TECHNIQUES IN THE CHARACTERIZATION OF SOMATIC CELL HYBRIDS	34
II. SPECIFIC TUMOR CELL CULTURES	44
A. HEMATOPOIETIC CELLS	44
B. LYMPHOID CELLS	54
C. NEUROBLASTOMA AND OTHER BRAIN CELLS	76
D. MAMMARY CELLS	86
E. INSULINOMA AND PANCREATIC CELLS	95
F. GASTROINTESTINAL CELLS	101
G. GENITOURINARY CELLS	105
H. MELANOMA AND SKIN CELLS	120
I. OTHER SPECIFIC CELL TYPES	124
III. SPECIFIC APPLICATIONS OF TISSUE CULTURE TECHNIQUES	129
A. EVALUATION OF DRUGS AND RADIATION DOSES IN CELL CULTURES	129
B. ANALYSIS OF TRANSFORMATION IN CELL CULTURES	136
C. GENETIC STUDIES OF CULTURED MALIGNANT CELLS	149
D. BIOCHEMICAL CHARACTERIZATION OF CULTURED TRANSFORMED CELLS	163
1. DNA AND RNA SYNTHESIS	163
2. AMINO ACID AND PROTEIN METABOLISM	169
3. BIOSYNTHESIS AND SECRETION OF HORMONES	177
4. UPTAKE AND TRANSPORT OF NUTRIENTS	181
5. GENETIC ANALYSIS OF BIOCHEMICAL EVENTS	184
6. OTHER METABOLIC ACTIVITIES	188
E. CULTURED CELLS AS A TEST SYSTEM FOR CHEMICAL CARCINOGENESIS	194
F. GROWTH STUDIES ON CELL CULTURES	204
G. OTHER SPECIFIC BIOLOGIC APPLICATIONS	215
IV. BROAD STUDIES ON IN VITRO TECHNIQUES	220

Investigator Index

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CURRENT CANCER RESEARCH

I. TECHNIQUES IN THE CHARACTERIZATION OF SOMATIC CELL HYBRIDS

A. GENETIC ANALYSIS OF HYBRIDS BY SOMATIC MAPPING AND MUTATION

1. COLLABORATIVE RESEARCH - MOLECULAR APPROACHES TO GENE MAPPING IN MAN

Chu, E. H., University of Michigan, Ann Arbor Campus, Medical School, Dept. of Human Genetics, *1335 E. Catherine St., Ann Arbor, Michigan*, 48104, United States of America

This award is for support of part of collaborative project concerned with the nature of gene regulation of differentiated functions. The technique of cell hybridization followed by analysis of specific functions, correlated with the presence or absence of specific chromosomes, is used primarily. The coordinated efforts of four laboratories are involved: Ruddle (Yale University), Skoultchi (Albert Einstein College of Medicine), Darlington (Cornell Medical School), and Chu (University of Michigan). The Yale lab is primarily involved in expanding the technological base of gene transfer experiments by building mouse chromosomes with selectable markers via microcell fusion and subchromosome exchange. The main focus of the lab at Einstein College of Medicine is a study of expression of RBC functions in hybrids of Friend erythroleukemic cells with non-erythroid cells, with special emphasis on functions that suppress globin expression. The Cornell lab is concerned with localizing and characterizing the genes governing the expression of serum proteins by measuring the effect of varying gene doses on expression of albumin, transferrin, and alpha-fetoprotein. The Michigan lab is primarily concerned with the production and analysis of new genetic markers and the development of improved techniques of gene mapping.

2. GENE MAPPING AND CHROMOSOME FRACTIONATION IN HUMAN TUMOR CELLS

Athwal, R. S., College of Medicine & Dentistry of New Jersey, New Jersey Medical School, School of Medicine, Dept. of Microbiology, *100 Bergen St., Newark, New Jersey*, 07103, United States of America

A combination of chromosome mediated gene transfer (CMGT) and microcell hybridization techniques will be used to transfer single human chromosomes individually into the Muntjac cells (2n equals 7).

The Chinese hamster hypoxanthine phosphoribosyl transferase (hprt) gene will be transferred to human hprt deficient cells and a large number of independent clones will be isolated. Following the integration of transferred hprt gene, single cell clones will be selected from independent clones to produce a series of cell lines, each carrying selectable markers (Chinese hamster hprt) integrated into a different human chromosome. The human chromosome carrying selectable markers will then be transferred into Muntjac cells by microcell fusion techniques. Hybridization of fractionated microcells provide a method to transfer a single or few chromosomes to a recipient cell. Thus, cell lines of Muntjac cells containing individual human chromosomes will be isolated.

The cell lines so produced will be used for gene mapping on human chromosomes. Because of the size differences between human and Muntjac chromosomes, it will be possible to separate a pure homogeneous population of human chromosomes present in Muntjac cells. DNA prepared for each human chromosome will be cloned in bacteria using phage lambda as a vector to develop clone libraries for each chromosome. The system provides a method to fractionate human genome into its components, 'the chromosomes.' The clone libraries can be further used to purify genes of interest.

3. TESTING DNA DAMAGE AND REPAIR WITH SOMATIC CELL HYBRIDS

Siniscalco, M., Petrovic, A., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This project focuses on some hitherto unexplored potentials of somatic cell hybridization to evaluate in strict genetic terms the biological effect of radiations and radiomimetic substances on the human diploid cells freshly derived from the propositi to be tested. The rationale of this methodology is essentially the same as that employed by Goss and Harris for the mapping of human genes with the so-called radiation co-transfer test. However, in order to make the test amenable to practical usage, we have restricted the screening for radiation damage to the small target site of the human X-chromosome long arm between Xq26 and Xqter which includes the loci for HGPRT and G6PD. White blood cells from normal individuals or from patients affected by or highly susceptible to various malignancies are submitted to a standard set of mutagenic treatments and then fused - by means of polyethylene glycol or Sendai virus - with rodent cells deficient in both HGPRT and G6PD. Preliminary studies have indicated that under these circumstances, actively growing hybrid cells isolated in the HAT selective medium within two weeks from the fusion experiment, have all regularly retained the human HGPRT while the retention of G6PD depends upon the co-transference of the unbroken target site into the hybrid genome. Thus the relative frequency of G6PD positive hybrid cells provides a quantitative measure of the radiation effect at the level of functional chromosomal DNA. Variations of this methodology will be applied to make it suitable also to the measurement of DNA repair. Depending upon the type of protocol and of the human material chosen, this new experimental procedure is expected to yield data on the individual variation in the susceptibility to DNA damage/repair and to offer an altogether new handle for the screening of hidden genetic damage in individuals chronically exposed (for whatever reason) to mutagenic carcinogenic agents.

4. MAPPING OF TUMOR VIRUS GENOMES IN TRANSFORMED CELLS

Croce, C. M., Botchan, P., Wistar Inst. of Anatomy & Biology, *36th & Spruce Sts., Philadelphia, Pennsylvania*, 19104, United States of America

It is the objective of this project: 1) To map the chromosomal site of integration of SV40 in different SV40 transformed human cell lines, 2) to establish the role of the SV40 A gene product in the proliferation of somatic cell hybrids between mouse peritoneal macrophages and SV40 transformed human cells, 3) to produce antisera for surface antigens coded by the human chromosomes carrying the SV40 genome, 4) to study the expression of SV40 T antigen in hybrids between mouse teratocarcinoma and SV40 transformed human cells, 5) to map the chromosomal site of integration of SV40 in different SV40 transformed mouse cell lines, 6) to map the transforming gene(s) of adenovirus 5 in adenovirus transformed cell lines, 7) to establish whether the genome of Epstein-Barr virus is chromosomally integrated in Burkitt's lymphoma cells derived from different patients; 8) to map the chromosomal site of integration of the murine sarcoma virus (MSV) genome in different MSV transformed human cells, 9) to map the sarcoma specific DNA sequences of Moloney sarcoma virus and of Kirsten and Harvey

sarcoma virus in normal mouse and rat cells respectively, 10) to map the structural genes for endogenous RNA tumor viruses in different mouse strains, and 11) to study the expression of malignancy in somatic cell hybrids between virus transformed and normal human cells.

5. ACTIVATION OF HEPATIC GENES IN CELL HYBRIDS

Darlington, G. J., Cornell University, Medical Center, Cornell Medical College, Dept. of Medicine, *1300 York Ave., New York, New York*, 10021, United States of America

Hybrids between mouse hepatoma cells and human leukocytes have been shown to secrete human serum albumin. I plan to use somatic cell hybrids between murine hepatoma lines and human diploid ones to map the structural loci for human albumin, transferrin, alpha-1 antitrypain, and ceruloplasmin. Other human cell proteins will be mapped using two-dimensional electrophoresis. The phenomenon of activation of human albumin will be studied to determine the kind of genetic components involved in expression of this human gene.

Variants of the hepatoma line that fail to secrete albumin will be used in complementation analysis to determine the number and kinds of loci governing albumin production. These same cells will be used to identify and map human genes involved in albumin secretion. Finally, embryonic cells at various developmental stages will be tested for their capacity to express organ specific functions following hybridization with differentiated hepatoma cells.

BIBLIOGRAPHIC REFERENCES: J.K. Rankin and G.J. Darlington. Expression of human hepatic genes in mouse hepatoma-human aminocyte hybrids. Somatic Cell Genetics, 1, 1-10, 1979. G.J. Darlington, H.P. Bernhard, R.A. Miller, and F.H. Ruddle. Expression of liver phenotypes in cultured mouse hepatoma cells. J. Natl. Cancer Inst. in press, 1980.

6. SOMATIC CELL GENETIC ANALYSIS OF X CHROMOSOME INACTIVATION - X CHROMOSOME INACTIVATION IN MOUSE X MARSUPIAL CELL HYBRIDS

Graves, J. M., Dawson, G. S., La Trobe University, School of Biological Sciences, Dept. of Genetics & Human Variation, *Bundoora, Victoria 3083, Australia*

OBJECTIVE: To study the mechanism of inactivation of X chromosomes in the somatic cells of female marsupials.

APPROACH: Work of Cooper, Sharman and Johnson has established that marsupials appear to have a different, perhaps ancestral, form of X chromosome inactivation from eutherians. Blood cells show paternal X inactivation, while cells of other tissues show partial or full expression of both X's. Conventional approaches to mapping the X are hampered by lack of polymorphisms for X linked genes - therefore an attempt will be made to use somatic cell genetic techniques to map the X. Using cells from females polymorphic for X linked genes, it will then be possible to construct cell hybrids in which re-expression of paternally derived isozyme variants is detectable. Thus, it should be possible to detect and map any control locus on the inactive X.

PROGRESS: Many mouse-marsupial cell hybrids have been obtained; these are unusal in that they contain only small fragments of marsupial chroeosmes, bearing the selected marker (Hpt) and some also flanking markers (PgK-A, Gpd). These markers have been mapped to the X in three species, and order tentatively established. To date, hybrids between mouse cells and cells from marsupial G6PD heterozygotes have expressed only the maternal G6PD, borne on the active X; no evidence has been obtained for re-expression of genes on the inactive X.

7. GENETICS OF SOMATIC CELLS

Siminovitch, L., Hospital for Sick Children, *555 University Ave., Toronto, Ontario*, M5G 1X8, Canada

Our laboratory is engaged in the characterization of mammalian cell function using genetic techniques and concepts. Until recently the approach has been to develop methods for the isolation of specific mutants involving enzymes or other cellular functions, and to examine these by biochemical-genetic techniques. Our present approach is to carry the analyses to further depth by DNA-mediated gene transfer techniques.

A number of mutants suitable for such gene transfer is available. These include dominant as well as conditional lethal isolates. As others have shown, we have successfully transferred the loci for thymidine kinase and methotrexate resistance by means of DNA. More recently we have also transferred loci for ribonucleotide reductase, asparagine synthetase and colchicine resistance. In order to generalize the system, we are developing a library of human DNA in an appropriate vector and this DNA, together with sib selection techniques, will be used in the future in an attempt to clone several mammalian genes.

8. HYBRIDIZATION, DNA FUNCTION, MUTATION IN CELL CULTURE

Littlefield, J. W., Felix, J. S., Johns Hopkins University, School of Medicine, Dept. of Pediatrics, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

The mechanisms causing cell variability in differentiation, development and neoplasia can be examined using the methods of somatic cell genetics. Our goal is to obtain new information about these mechanisms by using embryonal carcinoma cells capable of determination and subsequent differentiation in vitro - an exciting surrogate for the mammalian embryo. Our objectives are: 1) to look for restoration of the ability to differentiate to endoderm among several nullipotent embryonal carcinoma cell lines through various modes of communication with pluripotent cells or other nullipotent lines; 2) to isolate endoderm-deficient variants from the pluripotent 'nursed' embronal carcinoma cell line PSA1 and from the 'emancipated' lines H6 and OC15S1; 3) to construct complementation groups among multiple variants within each of these lines using hybridization and perhaps cybridization techniques; and 4) to investigate the biological and biochemical nature of the defects in these complementation groups. With these studies we hope to construct eventually a complementation map of the biochemical steps involved in the commitment of pluripotent embryonic cells to endoderm and to identify these individual steps.

9. SOMATIC CELL GENETICS OF DIFFERENTIATED CELLS

Chasin, L. A., Columbia University, Graduate School of Arts & Sciences, Dept. of Biological Sciences, *Broadway & W. 116th St., New York, New York*, 10027, United States of America

The regulation of expression of differentiated functions in a clonal rat hepatoma cell line will be studied. The basic approach is to isolate variant subclones that are altered in the expression of liver-specific functions. The goal is to explain in molecular terms the mechanism responsible for heritable variation in phenotype, be it genetic or epigenetic. The principal focus is on serum albumin production. We will study four subclones; three secrete albumin at different rates spanning a 100-fold range. The fourth produces no detectable albumin. These clones will be analyzed with respect to synthesis and secretion of albumin. The mRNA sequences for albumin will be quantitated and compared using a cloned cDNA probe. Nuclear, polysomal, and poly A containing fractions will be separately analyzed. In this way we hope to identify the level of variation distinguishing these four subclones.

Additional variant subclones altered in albumin production will be sought using immunochemical techniques to locate non-producing or overproducing colonies. Revertants of an albumin non-producing clone will be screened for in the same way. Electrophoretic mutants in the albumin gene will be sought using an isoelectric focusing screening method.

A second facet to this work is the isolation of variants deficient in the production of liver-specific enzymes normally found in the hepatoma cells.

Mutants lacking the liver form of alcohol dehydrogenase will be selected on the basis of their resistance to pentenol or other alcohols whose oxidation results in a toxicity product. The mutants will be screened for concomitant deficiencies in other liver specific phenotypes (tyrosine aminotransferase induction, albumin secretion) in order to define pleiotropic events affecting whole programs of genes for specialized functions.

In the third part of the project, the hormonal induction of alpha 2U globulin in a subclone of the hepatoma cell line will be studied. This liver specific protein is the major urinary protein in male rats. Induction by the glucocorticoid dexamethasone will be characterized with respect to hormonal specificity and requirements and the sequence of molecular events that com-

prise the induction process, including the synthesis and processing of mRNA sequences.

10. GENETIC ANALYSIS OF GENE REGULATION IN SOMATIC CELLS

Thirion, J. P., Universite de Sherbrooke, Centre Hospitalier Universitaire, Dept. of Microbiology, *Sherbrooke, Quebec*, J1H 5N4, Canada

The overall objectives are to study somatic cell genetics and elucidate regulation of Chinese hamster cells.

Mutants specific for the metabolism of alcohol will be isolated and characterized genetically and biochemically.

11. SOMATIC MUTATION AND CHEMICAL CARCINOGENESIS

Starkey, J., Washington State University, School of Veterinary Medicine, Dept. of Veterinary Microbiology & Pathology, *Pullman, Washington*, 99163, United States of America

OBJECTIVE: Demonstrate that polycyclic hydrocarbon carcinogens produce mutations in areas of the genome of mammalian cells in culture, which will render the cells more liable to further genetic change.

APPROACH: It will be demonstrated that these mutations are important in initiating carcinogenesis by showing that all clones with such mutations are tumorigenic on hamster cheek pouch inoculations, whereas those without such mutations are not. If successful this will provide a tissue culture model of a modified somatic mutation theory of carcinogenesis.

B. GENETIC ANALYSIS OF SOMATIC CELL HYBRIDS BY OTHER SPECIFIC TECHNIQUES

12. AN ANALYSIS OF TRANSFORMATION IN THE BLADDER USING CELL HYBRIDIZATION

Franks, L. M., Durbin, H., Imperial Cancer Research Fund, Cellular Pathology Laboratory, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

Cell hybridization is a valuable technique for analyzing functional changes in gene expression, particularly the expression of transformed characters. A major disadvantage of hybridization experiments in the past has been the need to use highly selected cell lines with built-in genetic markers that can be used to select against unfused parental cell or homokaryons. A second disadvantage has been the lack of normal epithelial cells for use in experiments concerned with the possible supression of 'malignancy' by normal cells. We have by-passed this second problem by using separated bladder and kidney epithelial cells (see Report 132). Two methods have been used for the selection of hybrids. In one, cells after fusion have been separated into fractions by size on an isokinetic gradient and the large cell fractions cloned. In the second method, each parental clone is treated with a different irreversible biochemical inhibitor (Wright, Experimental Cell Research 1978, 112, 395) so that only heterokaryons will survive. Using these methods we are trying to relate chromosomal changes with tumor production and other transformed characters in mouse and human tumor/tumor and tumor/normal hybrids (see Reports 143-145, 148).

13. INVESTIGATION OF VIRUS-RELATED NUCLEOTIDE SEQUENCES IN HUMAN NORMAL AND NEOPLASTIC CELLS

Jones, K. W., Kinross, J., Maitland, N., University of Edinburgh, Inst. of Animal Genetics, *W. Mains Rd., Edinburgh EH9 3JN, Scotland, United Kingdom*

OBJECTIVE: To determine the significance of virus-related nucleotide sequences in human tumors and normal tissues.

APPROACH: Nucleic acid hybridization in situ, DNA cloning and sequencing.

PROGRESS: Human uterine cervix dysplasias and carcinomas contain RNA related to herpes simplex virus type 2 and to adenovirus type 2, by in situ hybridization. Normal human tissues contain sequences related to Ad2 by blot hybridization.

14. GENETIC ANALYSIS OF THE 14Q(PLUS) TRANSLOCATION IN HUMAN LYMPHOMAS

Puck, T., Epstein, A. L., University of Colorado, Health Sciences Center, Eleanor Roosevelt Inst. for Cancer Research, *4200 E. 9th Ave., Denver, Colorado*, 80220, United States of America

This proposal is directed at studying the biological significance of the 14q(plus) chromosome abnormality described for African and non-endemic Burkitt's lymphoma and other human B-cell malignancies. By the use of somatic cell hybridization techniques, human-Chinese hamster ovary cell hybrids containing the 14q(plus) translocation will be prepared to enable the genetic products of this abnormal chromosome to be studied by immunologic characterization and two-dimensional polyacrylamide gel electrophoresis. By comparing these proteins to those of hybrid clones containing the normal chromosome 14 from the same patient, it is hoped that those proteins involved in the malignant process can be identified for further characterization. Since this chromosomal aberration has been correlated with the malignant state in a number of human B-cell tumors, an analysis of the molecular and genetic nature of the 14q(plus) translocation may lead to a better understanding of the neoplastic process and its relationship to differentiation. The technology gained from this investigation may be useful in the study of other human cancers such as chronic myelogenous leukemia which contains the Philadelphia chromosome.

15. GENETIC ANALYSIS OF THE PLASMA MEMBRANE IN MAN USING CELL HYBRIDS

Shows, T. B., Roswell Park Memorial Inst., Dept. of Experimental Biology, *666 Elm St., Buffalo, New York*, 14263, United States of America

A genetic analysis of the human cell surface will be accomplished at three levels: 1) external membrane proteins, 2) plasma membrane glycoproteins, and 3) membrane antigens. The external membrane proteins and glycoproteins will be labeled by standard procedures and analyzed by two-dimensional electrophoresis. Membrane antigens will be analyzed using the lymphocyte hybridoma strategy in which monoclonal antibodies are produced against human surface determinants. The expression of each of these three types of plasma membrane components will be ascertained on: 1) human cell lines derived from different tissues, 2) human cell lines from individuals with inherited metabolic diseases in which the plasma membrane may be altered, 3) transformed human cell lines, and 4) neoplastic human cell lines. The normal and abnormal electrophoretic and/or antigenic phenotypes determined for the plasma membrane components on these different cell types will be investigated in rodent-human cell hybrids. After fusion of the different human cell types with mouse cells the somatic cell hybrid methodology will permit dissecting the components of the plasma membrane and the determination of chromosome assignments of genes coding for human plasma membrane proteins, glycoproteins, and antigens. This research will characterize the plasma membrane phenotypes of a variety of different human cell types, determine if the phenotypes are of a genetic origin, and map human plasma membrane genes. Such information is important for understanding the genetic organization and biogenesis of the cell surface.

16. GENETIC ANALYSIS OF CELL HYBRIDS

Jonasson, J. A., Uppsala Universitet, Inst. of Medical Genetics, *Vastra Agatan 24, 75220 Uppsala, Sweden*

OBJECTIVE: The purpose of this project is to find a genetic correlative with tumorigenicity.

APPROACH: Working with an in vitro system of normal diploid cells, malignant melanoma cells (PG19) and cell hybrids, we are currently engaged in a study of the segregation of mitochrondrial DNA upon cell fusion.

PROGRESS: The most recent results of this project will shortly appear as 'Analysis of sister-chromatid exchanges and tumorigenicity in cell hybrids' (J. Cell Sci., in press).

17. INTEGRATION OF BABOON TYPE C VIRUS IN HUMAN X RODENT HYBRID CELLS

Lemons, R., Sherr, C. J., Cohen, C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Viral Pathology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Somatic Cell Hybrids derived by fusion of either mouse or hamster cells with human cell lines were infected with baboon endogenous type C virus (BEV) and scored for virus production. The human and rodent chromosomal content of hybrid cells was identified using assays for 30 gene-enzyme systems

(isozymes) representing 22 human chromosomes. A concordance between BEV expression and the presence of human chromosome six was observed (97% synteny in 160 analyzed clones) defining a new genetic locus (Bevi) required for productive BEV infection. Southern blot analyses of integrated proviral DNA in hybrid clones showed that (1) cells segregating human chromosome six also segregated the integrated BEV provirus and (2) BEV is integrated at random molecular sites in human DNA. Thus Bevi is either a preferred complex locus for integration of BEV in human DNA or, alternatively, a regulatory gene providing functions critical for maintaining the integrated BEV provirus.

18. ANALYSIS OF MALIGNANCY BY CELL FUSION

Harris, H., Bramwell, M. E., Rodgers, A., Wouters, M., Gingrich, R., University of Oxford, Sir William Dunn School of Pathology, *S. Parks Rd., Oxford OXI 3RE, England, United Kingdom*

OBJECTIVE: Cell fusion techniques are being used to determine the number and nature of the genetic lesions determining the malignant phenotype.

APPROACH: Malignant cells are fused with normal diploid cells and with each other and the phenotypes of the hybrid cells and segregants derived from them are examined.

PROGRESS: Substantial progress has been made in determining the number and type of lesions involved in determining the ability of cells to grow progressively in vivo. Of special interest is the recent finding that malignancy is associated with an abnormality in one of the proteins of the cell membrane.

19. GENE REGULATION IN DIPLOID AND HYBRID MAMMALIAN CELLS

Miller, O. J., Columbia University, College of Physicians & Surgeons, Dept. of Human Genetics & Develop./Obstetrics & Gynecology, *630 W. 168th St., New York, New York*, 10032, United States of America

The object of this study is to understand the regulation of ribosomal RNA synthesis and nucleolus organizer (NOR) activity in interspecific somatic cell hybrids and in amplified ribosomal RNA gene clusters. Suppression of these functions will be looked for in heterokaryons both in heteroploid x diploid and diploid x diploid somatic cell hybrids in an attempt to define the mechanism of suppression. We will evaluate the role of specific human or mouse chromosome loss in species-specific suppression of ribosomal RNA gene activity in hybrids that lose either human or mouse chromosomes. We will determine whether ribosomal RNA genes in interspecific hybrids and in amplified gene clusters are inactivated by enrichment of the DNA sequences for 5-methylcytosine, and whether viral infection or transformation will lead to undermethylation and gene reactivation. Reactivation of inactive NORs in hybrid cells will be attempted by back-hybridization to one of the parental cell types containing distinguishable NOR chromosomes.

20. MOUSE TERATOCARCINOMA - A SYSTEM FOR THE STUDY OF MAMMALIAN DIFFERENTIATION AND NEOPLASIA

Illmensee, K., Duboule, D., Illmensee, R., Petzoldt, U., Universite de Geneve, Lab. of Cell Differentiation, Dept. of Animal Biology, *154 Rte de Malagnou, CH1224 Geneva, Switzerland*

OBJECTIVE: To study xenogeneic gene expression and the developmental potential of malignant hybrid cells in chimeric mice.

APPROACH: Malignant hybrid cells of rat hepatoma x mouse teratocarcinoma origin were injected into C57BL/6 mouse blastocysts which subsequently were transferred into the uteri of pseudopregnant ICR females. Fetuses at days 15-18 were autopsied and analyzed for hybrid cell derived tissue contributions. In all four chimeric fetuses the hybrid cell participated in liver differentiation and also contributed to a limited number of organs of endomesoderm origin. The presence of rat chromosomes was confirmed by karyotypic analysis of the chimeric organs. Rat gene expression in these chimeric tissues was demonstrated by the presence of rat-specific enzyme variants and proteins, revealed by use of acetate and 2-D gel electrophoresis. The appearance in vivo of certain rat-specific gene products that were not found in the hybrid cells under conditions in vitro as well as tissue-specific modulation of enzyme patterns documented functional gene expression of the introduced xenogeneic chromosomes. After subcutaneous injection of these chimeric tissues into nude mice about one third of them developed tumors with a cellular and biochemical phenotype similar to, but not identical with, the hybrid cell tumors. These results indicate that rat x mouse hybrid cells are capable of participating in organ differentiation but also retain their malignant properties to some extent.

(This research is carried out in collaboration with Dr. C. Croce, Wistar Institute, Philadelphia, PA 19104, USA.)

21. CHROMOSOME LOSS FROM SOMATIC CELL HYBRIDS

Graves, J. M., La Trobe University, School of Biological Sciences, Dept. of Genetics & Human Variation, *Bundoora, Victoria 3083, Australia*

OBJECTIVE: To study the causes of chromosome segregation from cell hybrids.

APPROACH: Polyploid derivatives of mouse and Chinese hamster cell lines have been obtained. Cell hybrids have been obtained from fusions between each combination of mouse and hamster cells, and their chromosome constitutions studied by C- and G-banding techniques.

PROGRESS: In the hybrids already characterized, it appears that the extent of hamster chromosome loss depends on the ratio of parental mouse and hamster chromosomes. Recent results from polyploid hamster x diploid mouse hybrids suggest that the direction of segregation may also be reversed if the input ratio of hamster to mouse chromosomes is high enough.

22. ANALYSIS OF MALIGNANCY BY MICROCELL-MEDIATED CHROMOSOME TRANSFER

Cowell, J. K., Southgate, J., Imperial Cancer Research Fund, Cellular Pathology Laboratory, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

Cell lines derived from mouse bladder epithelium show consistent minimal deviations from the normal karyotype (Report 141). These findings are consistent with the view that chromosomal changes may be involved with the expression of the malignant phenotype. Various laboratories have shown that fusion of normal and tumor cells usually results in suppression of the malignant phenotype in the hybrids. Tumorigenic variants from these cells have lost chromosomes. Karyotypic analysis of these variants can be used to assess the role of specific chromosomes in malignancy. Analyses of this kind are complicated by random chromosome loss from the hybrids and the possibility that malignancy may result from the interaction of several chromosomes.

The use in fusion experiments of microcells, which contain one or a few chromosomes, may overcome these problems. Microcells have been prepared from mouse cells using colchicine and cytochalasin B treatments (Fournier and Ruddle, Proceedings of the National Academy of Sciences 1977, 74, 319). We are attempting to cross microcells from normal mouse cells with tumor cell lines and vice versa to assess the importance of individual chromosomes in the expression of the malignant phenotype.

23. MOUSE TERATOCARCINOMA, MAMMALIAN DIFFERENTIATION AND DISEASE

Illmensee, K., Bluthmann, H., Burki, K., Petzoldt, U., Universite de Geneve, Dept. of Biology, *154 Rte de Malagnou, CH1224 Geneva, Switzerland*

OBJECTIVE: The aims of our program are to cycle malignant cells through mice to identify the developmental basis of genetic defects or neoplasia and to gain insight into the mechanisms controlling gene activity during mammalian differentiation. Specific emphasis is focused on the in vivo potential and the reversibility versus stability of malignancy of various cancer cells.

APPROACH: Malignant cells from various mouse teratocarcinomas, human x mouse or rat x mouse hybrid cell lines are injected into mouse blastocysts carrying several genetic markers. After micromanipulation, the blastocysts are surgically implanted into the uterus of pseudopregnant foster mothers to allow development to term. The live-born mice are analyzed for tumor cell-derived contributions in the coat, internal organs, and germ line using enzyme electrophoresis, histochemical and histological techniques, and breeding tests.

PROGRESS: Hybrid cells between mouse teratocarcinoma, deficient in thymidine kinase, and human fibrosarcoma or

rat hepatoma, deficient in hypoxanthine phosphoribosyltransferase, lost their malignant properties after microinjection into C57BL/6 blastocysts and contributed normally in differentiation of several adult tissues. Human-specific galactokinase could be detected in two different organs of two chimeric mice. Nine rat-specific enzymes were present in a limited number of tissues of three chimeric mice. The in vivo appearance of certain rat gene products, usually not found in the hybrid cells in vitro, documented functional expression of the xenogeneic genes.

24. MODULATION OF GENE PRODUCT IN MOUSE/ CHICK ERYTHROCYTE HYBRIDS

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This is part of a broader project. A summary of this subproject is not available.

C. IDENTIFICATION OF HYBRID REGIONS IN SOMATIC CELLS BY IMMUNOLOGIC TECHNIQUES

25. PRODUCTION OF MONOSPECIFIC LYMPHOMA ANTIBODIES

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OBJECTIVES: Attempt to produce xenogeneic monospecific antibodies to human lymphoma and leukema associated antigens. Attempt to fuse antibody producing cells of mouse origin with mouse myeloma cells in order to produce hybrid cell lines capable of continuous production of monospecific antibody against tumor-associated antigens in vitro. Compare two hybrid techniques. One method to be compared will involve the hybridization of preselected clones using a method developed by Klinman. The second method will involve hybridization of immune spleen cells from whole spleens of immunized mice, then cloning the resulting hybrids, and finally searching for monospecific antibody. Also, evaluate the specificity of the presumed monoclonal antibodies and investigate the pattern of antigenic tumor-specific determinants on malignant lymphoid cells.

26. GENETIC AND BIOCHEMICAL ANALYSIS OF HUMAN LYMPHOID CELL SURFACE ANTIGENS

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The genetic and biochemical analysis of human cell surface antigens has been made possible by the techniques of somatic cell hybridization. The dominant expression of human cell surface structures on human x mouse cell hybrids has allowed the mapping of human surface antigens to specific human chromosomes. More recently, the ability to generate, using the method of Milstein, monoclonal antibodies to cell surface structures has facilitated their biochemical characterization.

We propose to begin a genetic and biochemical study of human lymphoid cell surface antigens. Xenogeneic (cross-species) monoclonal antibodies to human lymphoid cells will be generated and screened by an indirect binding assay. Standard antibody fluorescent techniques will be used in conjunction with a fluorescence-activated cell sorter to separate antigen positive cells in human x mouse cell hybrids. Karyotypes of these cells will be examined to correlate specific human chromosomes with antigen expression. Through biochemical labelling techniques followed by monoclonal antibody immunoprecipitation, these antigens will also be characterized as to molecular weight and subunit structure.

The study of cell surface antigens of human lymphoid cells may help define functionally and developmentally distinct subsets of lymphoid cells. This information may lead to the clinical management of immunological diseases and neoplastic progression. Moreover, the mapping of the genes determining these cell surface structures will contribute to our knowledge of the organization of human chromosomes.

27. ANTIGENICITY AND TUMORIGENICITY OF SOMATIC CELL HYBRIDS

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This proposal is predicated on two properties of human-murine somatic cell hybrids: the gradual loss of human chromosomes, allowing for clonal isolation of hybrids with one or few human chromosomes; and the co-dominant expression of constitutive cell surface antigens of each parent on the cell surface. Hybrid cells containing one or several human chromosomes will be injected into mice syngeneic to the mouse parental cell, and the antisera analyzed on panels of target cells designated so that reactions specific to human cell surface molecules will be demonstrated. Immunoprecipitation and electrophoretic analysis will be used to discriminate the number of antigenic molecules coded for by a particular chromosome and these antisera will be used for biochemical and functional analysis of the molecules themselves. Humoral reactivity to tumorigenic (in the nude mouse) hybrid cells derived from fusions of human tumor-derived and normal mouse cells will be analyzed to determine if tumor-associated specific antigens unique to the human parental tumor-derived cell lines and tumor cell lines of similar histologic type are expressed on these hybrids. We shall also screen these antisera for reactivity to human and/or mouse embryonic antigens which are often re-expressed on the tumor cell surface.

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28. HYBRIDS OF HUMAN HEPATOMAS AND MOUSE TERATOCARCINOMAS

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The proposed project involves the hybridization of murine embryonal carcinomas (ECC) with a recently isolated human hepatoma, which is unique in its capacity to synthesize and secrete a wide range of plasma proteins. Based on the results of similar fusions with ECC, there are several possible outcomes that would be of interest for further investigation. If the differentiated hepatoma phenotype is extinguished and the ECC characteristics are maintained, such hybrids might be induced to differentiate by retinoic acid. We would then determine if any aspects of the hepatoma parent reassert themselves. We would look for the disappearance and reappearance of human HLA antigens, to see if they were co-regulated with the murine H-2. Similarly, we would test for the reexpression of plasma protein synthesis, by established immunologic and electrophoretic techniques, and also check for the appearance of murine versions of these proteins, in case the differentiation of the ECC was 'guided' by the hepatoma parent. If the differentiated hepatoma characteristics are never extinguished in hybrids, the latter possibility will also be tested; further, such hybrids will be used to map plasma proteins to specific human chromosomes. This may be facilitated by immunoselection with existing chromosome-specific monoclonal antibodies against surface antigens.

29. EXPRESSION OF TUMOR-ASSOCIATED ANTIGENS ON SOMATIC CELL HYBRIDS BETWEEN MALIGNANT AND NORMAL CELLS

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OBJECTIVES: To determine expression of tumor-associated antigens on somatic cell hybrids of malignant cells with normal partner cells. To obtain tumor cell hybrids having the ability to induce specific immunity against the parental tumor cell in host animals.

APPROACH: Tumor-associated surface antigen (TASA) has been detected on mouse ascitic tumor cells derived from spontaneous mammary carcinoma. The antigen has a potent antigenicity to induce humoral response which specifically protects syngeneic recipient against antigen-bearing tumor. After cell hybridization of ascitic tumor cells with 8-azaguanine resis-

tant L cells by using UV-irradiated Sendai virus, hybrids were selected and cloned in HAT medium. Expression of TASA and H-2 antigen was determined by mixed hemadsorption test. Karyological analysis was also done. Tumorigenicity was tested by inoculating an appropriate number of hybrid cells into irradiated syngeneic animal. Immune resistance after inoculation of hybrid cells or irradiated tumor cells was measured by take incidence of lethal tumor challenge.

PROGRESS: Immune resistance against parental tumor after tumor hybrid inoculation was remarkably superior to that after inoculation of irradiated tumor cells. It was due to cytotoxic splenic T cells evoked by tumor hybrid inoculation. Constellation of surface antigens on tumor hybrid cells should be clarified as compared with those on parental tumor cells.

30. TUMOR AND DIFFERENTIATION ANTIGENS IN THE IMMUNE CONTROL OF NEUROBLASTOMA

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OBJECTIVE: To isolate and characterize structural antigens responsible for the specific anti-tumor immune response in neuroblastoma (NB) tumor-bearing A/J mice.

APPROACH: 1) Isolation of clones and somatic cell hybrids of NB which are high-, low-, or non-producing for specific alloantigenic structures; 2) isolation and immunochemical characterization using monoclonal antibodies of tissue-type and tumor-associated antigens from cloned NB cells; 3) protection of syngeneic mice against NB cell challenge by inoculation of individual NB-associated antigens.

PROGRESS: Various clones and hybrids of the C1300 NB cell line which differ in particular structures antigenic in a syngeneic system have been isolated. In addition, a new line of neuroblastoma, NB-SV, has recently been obtained by infecting embryonic sympathetic neurons from strain A mice with SV40 virus. Antigens have been solubilized from cloned NB cells and partially purified. These fractions retained antigenicity as defined by a variety of immunological tests, including radioimmunological methods using antibodies from syngeneic mice immunized against fixed NB cells or NB cell membranes. Monoclonal antibodies anti-NB have been obtained by somatic cell fusion between plasmacytoma cells, P3x63Ag8, and spleen lymphocytes from A/J mice a) NB tumor-bearing, b) immunized against NB cell membranes. The specificity of these antibodies is being investigated using purified C-type RNA-viral antigens and the solubilized fractions from NB cells.

31. SEROLOGIC ANALYSIS OF THE T/T LOCUS USING SOMATIC CELL HYBRIDS

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The primary objective of the proposed experiments is the establishment of malignant cell lines expressing the products of wild-type and mutant t alleles. Two techniques to establish such cell lines are described. These techniques are based on available evidence that teratocarcinoma-derived embryonal carcinoma cell lines allow the genotypic expression of T/t-complex products. The first method described is the somatic cell hybridization of t-factor positive embryo cells with enzyme-deficient embryonal carcinoma cells to produce clonal cell lines. The second method proposed utilizes established techniques of teratocarcinoma induction; t-factor positive teratocarcinoma cell lines will be derived by grafting t-factor positive embryos into the testes of syngeneic males.

32. ANALYSIS OF CELLULAR RESISTANCE TO ICRF 159 BY SOMATIC CELL HYBRIDIZATION

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Two sublines of BHK 21S cells with independently induced resistance to the anti-tumor agent ICRF 159, BS/159-1 and BS/159-4, have been fused to drug-sensitive cell lines and the nature of the cellular resistance has been studied by characterizing the drug response of the resultant hybrids in colony-forming assays.

Intraspecific hybrids between the wild-type BHK 21S cells of Syrian hamster origin and each of the two resistant lines demonstrate co-dominant expression of resistance to ICRF 159. Interspecific hybrids from fusions between the resistant lines and mouse 3T3 cells also express drug resistance but to a much lesser extent than the intraspecific hybrids.

In order to investigate this difference still further we have used BHK/C13 Syrian hamster cells, which share certain relevant growth characteristics with the 3T3 cells, as the sensitive line in a subsequent series of intraspecific fusions. The hybrids derived from crosses with the resistant lines show a drug response consistent with the pattern seen in the intraspecific hybrids where BHK 21S cells were used as the sensitive line.

It seems possible therefore that the lesser manifestation of drug resistance in the interspecific hybrids may be due to preferential expression of a mouse function involved in the response to ICRF 159. We are now attempting to derive human cell lines with induced resistance to ICRF 159 in order to study the expression in cell hybrids and any possible correlations with human chromosome loss in human/mouse hybrids.

33. USE OF SPECIFIC ANTIBODIES TO IDENTIFY RNA-DNA HYBRID REGIONS

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This is part of a broader project. A summary of this subproject is not available.

D. OTHER SPECIFIC TECHNIQUES IN THE CHARACTERIZATION OF SOMATIC CELL HYBRIDS

34. CLONAL ANALYSIS OF TUMORIGENICITY IN HUMAN BLADDER TUMOR CELL LINES USING CELL HYBRIDIZATION

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The relationship between tumorigenicity, transformed phenotype and chromosome constitution is being investigated using somatic cell hybridization. The first of these experiments has involved two tumorigenic diploid clones, 8D and beta, derived from the human bladder tumor cell-line, EJ. The clones vary in their chromosome constitution; clone 8D has 46 chromosomes and clone beta, 47. The clones differ in their morphology and have different marker chromosomes.

The cells were fused with Polyethyleneglycol 100 and separated on an isometric Ficoll gradient. Ninety-two clones were analyzed chromosomally and the diploid clones discarded. Of the 35 tetraploid clones analyzed, 7 are heterokaryons (8D/beta), 10 are homokaryons (8D/8D) and 1 is a hexaploid heterokaryon (8D/beta/8D). The morphology of these clones varies; 5D is particularly distinctive with large multinucleate cells. The tumorigenicity in nude mice of the 4 hybrids tested so far is as follows: 1D(8D/beta) - 0/3; 4F (8D/8D) - 3/3; 5D (8D/beta/8D) - 1/3; 11E (8D/beta) - 3/3.

Chromosome analysis of the cultured tumors is identical to the original cell inoculum.

Detailed analysis of these clones and tumors and hybridization of near-diploid tumor- and non-tumor-producing EJ clones and normal epithelium and mesenchyme are now in progress.

This project summary contained formulas, drawings, tables or nonkeyable data which are not shown above.

35. ANALYSIS OF SALIVARY GLAND TRANSFORMATION IN VITRO USING CELL HYBRIDIZATION TECHNIQUES

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A number of studies on hybrids constructed between normal and malignant cells have shown that the presence of chromosomes from the normal parent can suppress expression of the malignant phenotype in the hybrid. We are in the process of constructing intraspecific hybrids between salivary epithelial cells at various stages in the transformation process - normal cells, cells from preneoplastic foci in primary culture and cells

from preneoplastic and carcinoma-producing cell lines. We plan to analyze the ability of a range of hybrids to produce tumors in vivo and correlate this with other parameters such as expression of in vitro markers of transformation, growth in agar in particular, and chromosome complement. It will not be possible to determine from which parent individual normal chromosomes were derived but the role of marker chromosomes characterizing some of the cell lines can be assessed.

Since none of our epithelial cell lines carry selectable genetic markers, we have used a method whereby parental cells are treated with a lethal dose of different biochemical inhibitors immediately before fusion with polyethylene glycol (Wright, Experimental Cell Research 1978. 112, 395). We treated cells in suspension with iodoacetamide (3-5mM) or diethylpyrocarbonate (0.005-0.0075 percent) for 30 minutes on ice. This was followed by fusion, using 40 percent PEG 1000 added to the pellet of pooled cells that had been pretreated with 100 micrograms/ml phytohemagglutinin. This enhances fusion efficiency and reduces the toxicity found at higher concentrations of PEG (Mercer and Schlegel, Experimental Cell Research 1979, 120, 417). Provided that toxicity in unfused parental cells or in homotypic fusions is monitored in each experiment, the probability that dividing colonies are derived from heterotypic fusions is near 100 percent. Identification of marker chromosomes in presumptive hybrid clones can confirm their origin.

36. CELL HYBRIDIZATION STUDIES ON THE HORMONAL REGULATION OF BREAST TUMOR CELL GROWTH IN CULTURE

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OBJECTIVE: Approximately one in three human breast tumors is hormone responsive, i.e., the growth of the tumor and its metastases is stimulated by circulating hormones. By blocking the action of these hormones it should be possible to slow if not prevent the growth of such tumors. We hope to gain new informaton about the steps involved in the growth response of tumor cells to hormones, their genetic regulation and the interactions of different classes of hormones with the same target cell. It is hoped that such infomation may contribute to the development of improved methods of endocrine therapy for tumors.

APPROACH: The technique to be used involves the construction of hybrid cells from cytochalasin B enucleated cells and isolated nuclei. Hybrid cells will be constructed using cytoplasm and nuclei from hormone responsive and unresponsive cells. The hormone responsive cells to be used are MCF-7 cells, a line of human breast cancer cells whose growth is promoted by estrogen, insulin and thyroxine. The response of the hybrid cells to various hormones will be examined.

37. HUMAN LYMPHOBLAST-MOUSE CELL HYBRIDS

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This is part of a broader project. A summary of this subproject is not available.

38. EFFECT OF ENVIRONMENTAL PH ON CELL FUSION IN HYBRID CELLS (HUMAN, HAMSTERS)

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This is part of a broader project. A summary of this subproject is not available.

39. HYBRIDOMAS - PRODUCTION AND GENETIC APPLICATIONS

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We are producing a collection of continuous hybrid cell lines secreting monospecifc antibodies of selected and defined specificities, namely antibodies against a series of well defined haptens and against human cell surface antigens. These hybridomas are made between mouse myelomas and mouse spleen cells. The antibodies will be used to study the genetics of mouse B cell differentiation and immunoglobulin production. The mouse B cell repertoire and its development from 'germ line' genetic information will be analyzed. Antibodies against human tumor antigens and differentiation antigens and possible cystic fibrosis specific products will be used to detect and purify these antigens and to analyze their expression on mouse-human hybrids. Those directed against antigens such as HLA will be made available for human lymphocyte typing. If antibodies against cystic fibrosis specific products are produced they will be available for detection of C.F. heterozygotes.

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40. MICROSURGICAL STUDIES OF NORMAL AND MALIGNANT TROPHOBLASTS

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OBJECTIVES: To microsurgically produce, culture, and subsequently analyze the biologic, cytogenetic, and endocrinologic characteristics of both intraspecific and interspecific chimaeras and somatic cell hybrids in vitro and in vivo in order to: 1) attempt to determine the control mechanisms of normal and malignant trophoblasts; 2) provide biologic and cytogenetic information for a greater understanding of tumor-host interaction and suppression of the tumorous state.

APPROACH: Direct recovery of blastocysts from pregnant hosts; explanation of normal murine mural trophoblast; use of established cell lines; microsurgical production of chimaeras and somatic cell hybrids using the methods of Gardner and Diacumakos, respectively; histologic, cytogenetic, and endocrinologic analysis of chimaeras, hybrids, and tumors by routine tissue section, chromosome banding, and radioimmunoassay.

PROGRESS: Serial passage of choriocarcinoma within the hamster cheek pouch; primary culture of choriocarcinoma with cytogenetic and endocrinologic analysis done in collaboration with Dr. Diacumakos and Dr. Jones, the Rockefeller University and the Memorial Hospital; microsurgical production of intraspecific and interspecific somatic cell hybrids and isolated murine trophoblastic vesicles and inner cell masses.

41. DEVELOPMENTAL AND MALIGNANT PROPERTIES OF TERATOCARCINOMA CELL HYBRIDS

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This project represents an approach to investigate the intracellular processes involved in the differentiation of murine embryonal carcinoma cells, the stem cells of teratocarcinomas. The embryonal carcinoma cells are developmentally multipotent and malignant whereas their differentiated progeny are benign.

The approach is to make use of embryonal carcinoma cell cultures and to apply to this system some of the techniques currently available for the genetic manipulation of cells in culture.

Cell hybrids have been isolated following fusion of embryonal carcinoma cells to committed erythroid cells. These hybrids resemble their erythroid parent in their ability to synthesize hemoglobin. I intend to continue investigation of these hybrids to determine the status of the globin genes derived from the embryonal carcinoma parent.

42. CYCLIC NUCLEOTIDE METABOLISM IN SOMATIC CELL HYBRIDS

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This is part of a broader project. A summary of this subproject is not available.

43. SOMATIC CELL HYBRIDIZATION

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This is part of a broader project. A summary of this subproject is not available.

II. SPECIFIC TUMOR CELL CULTURES

A. HEMATOPOIETIC CELLS

44. STUDIES ON PROLIFERATION AND DIFFERENTIATION IN HUMAN LEUKEMIA

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OBJECTIVE: The aim is to study the normal regulation of granulopoiesis, to study the regulation of cell production in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), and, in particular, the interaction between leukemic and normal hemopoieses. We also intend to follow the evolution in CML and with the use of clonogenic techniques find prognostic parameters in leukemia.

APPROACH: The agar culture technique is used for detection of granulopoietic stem cells. Cell differentiation is studied in diffusion chamber in the peritoneal cavity of mice. We try to detect factors produced by normal or leukemic cells, which have an inhibitory effect on normal and leukemic stem cells grown in vitro. These factors are characterized biochemically and, hopefully, purified for studies of the mechanism of action and eventually for development of radioimmunoassays in order to detect these factors in sera of leukemic patients. Sequential studies of the in vitro growth pattern in agar are performed in AML and CML to observe the evolution of malignancy, the interaction between normal and leukemic cells and prognostic parameters.

PROGRESS: A low molecular weight granulocyte derived inhibitor of granulopoietic stem cells has been found. Similarly, a high molecular weight leukemia associated inhibitor of normal granulopoietic stem cells was found. The latter may be of importance for suppression of hemopoiesis in leukemia. Distinct differences in diffusion chamber growth were found in chronic and acute phase CML as well as between spleen and marrow cells in CML.

45. BONE MARROW CULTURE IN AGAR-GEL - A SEARCH FOR ETIOLOGIC FACTORS IN LEUKEMIA

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The aim of these studies is to characterize the cellular and humoral events regulating granulocyte production and to determine whether alterations of these regulatory steps can be defined in human hematopoietic neoplasms and exploited to increase understanding of the pathophysiology and treatment of these disorders. Previous studies have indicated that lymphocytes, monocytes, and microorganisms are involved in colony stimulating factor (CSF) production and granulocyte regulation. The nature of the lymphocyte-to-monocyte step has not been characterized. One of the aims of the present work will be to determine whether this requires cell-to-cell interaction or is the result of a humoral factor and to further characterize the role of microorganisms in CSF and granulocyte production. Serial colony stimulating activity (CSA) determinations will be carried out in infected and burned humans and the level found correlated with peripheral blood neutrophil counts and evidence of infection. Viruses, particularly influenza and herpes viruses, have been found to inhibit CSF production in vitro. Studies will be conducted to determine the nature of this observed effect. Attempts will be made to determine whether a specific eosinophil CSA can be produced in vitro by incubating sensitized normal human mononuclear cells with ragweed pollen and other antigens. Serial studies will be carried out to determine whether changes in bone marrow CFU-C numbers can be determined in human preleukemia, which can predict the eventual outcome of this disease. The stability and viability of bone marrow obtained from patients with AML and CML in remission and cryopreserved at minus 178 degrees for autologous transplantation will be investigated by studying CFU-C content at serial time points. The effect of plant hormones on colony formation and cellular maturation of normal human myeloid and leukemic cells will be studied. Attempts will be made to enucleate normal human myeloid and leukemic cells for subsequent studies of nuclear cytoplasmic transfer.

46. BONE MARROW CULTURE IN ACUTE MYELOID LEUKEMIA

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OBJECTIVES: 1. To assess the role of stimulators of granulopoiesis in determining the response to induction chemotherapy in acute myeloid leukemia. 2. To analyze the changes in physical and functional properties of normal granulocyte-monocyte progenitor (clone-forming) cells which occur as these cells differentiate down the granulocyte-monocyte pathway and to compare these with the properties of equivalent AML clone-forming cells in order to distinguish genuine abnormalities of the leukemic cells from properties reflecting differences in maturity between AML and normal clone-forming cells.

APPROACH: 1. In vitro techniques will be used to assess the responsiveness of leukemic cells to colony stimulating factors (CSF) and to assay CSF levels in leukemic patients both before and during therapy. Response to agents known to increase CSF will be monitored. 2. Assays of responsiveness of cells will be used in combination with cell separation techniques which exploit physical and functional properties to achieve separation.

PROGRESS: This is a new project.

47. CHEMOTHERAPY OF NORMAL AND LEUKEMIC MARROW IN VITRO

Park, C. H., Hoogstraten, B., Amare, M., University of Kansas, Medical Center, School of Medicine, Dept. of Medicine, *39th St. & Rainbow Blvd., Kansas City, Kansas,* 66103, United States of America

We have developed a new cell culture method (Feeding Culture) for colony formation human leukemic marrow cells (L-CFU), which can be used as a quantitative assay for leukemic stem cells. With the previous grant from USPHS, we have solidified the value of this culture assay by a number of quantitative (e.g., successful colony growth, over 73% in 80 patients with acute nonlymphocytic leukemia), and qualitative studies (e.g., chromosome analysis). Our preliminary study in a limited number of patients on the in vitro chemotherapy effect on L-CFU in comparison with that on CFU-C (normal granulocytic progenitor cells) showed an extremely good correlation with clinical chemotherapy response (p less than 0.001). We plan to extend this study to a larger number of patients with a variety of in vitro chemotherapy exposures (change in schedules and drug combinations, and trials of new drugs, etc.) in order to find the best in vitro predictive system for clinical chemotherapy response and also to find new improved chemotherapy regimens. Cell-sorting by flow microfluorometry with the use of supravital DNA stain (H33342) followed by Feeding Culture for L-CFU with or without in vitro chemotherapy exposure will be done in order to improve understanding of leukemic stem cell kinetics and to facilitate the development of the new improved chemotherapy regimens. We will characterize and quantitate the effects of in vitro growth regulating factors (L-ascorbic acid, colony stimulating factor and leukemic suppressing factor) found to be important for L-CFU. This will enable better utilization of our culture assay and may also lead to new mode of controlling leukemia.

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48. REGULATION OF ERYTHROID DIFFERENTIATION IN NORMAL AND LEUKEMIC HEMATOPOIETIC CELLS

Deisseroth, A., Colbert, D., Hendrick, D., Bode, U., Fontana, J., Wright, D., Maniatis, T., Kan, Y., Stammatoyannopoulos, G.,

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The genetic mechanisms governing hematopoietic differentiation in normal and leukemic erythroid and myeloid cells are being studied through use of cell lines which contain markers of human hematopoietic differentiation. Hybrid mouse erythroleukemia cell lines which contain the chromosome bearing the human alpha globin genes as the only human chromosome have been derived using human donor cells which differ with respect to their epigenotype, stages of development and genetic composition. A series of such hybrid cells, each containing human alpha globin genes from patients in whom non-deletion mutations have led to altered alpha globin gene expression, has been constructed. The various steps of gene expression are being characterized using Southern and northern nucleic acid transfers as well as 2 dimensional gel chromotography of non-histone chromosomal proteins. Similar techniques are being applied to HL60, a continuously proliferating human promyelocytic leukemia cell line, in hopes of understanding the regulation of markers of monocyte and of myeloid differentiation in these human leukemia cells, as well as the process which governs commitment to normal maturation in normal cells which is defective in the HL60 cells.

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49. HUMAN LEUKEMIA MARROW

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The mechanism of bone marrow failure in acute leukemia is addressed in this research project. When short-term tissue culture techniques are used, bone marrow cells from normal individuals or patients with acute leukemia in remission respond in a characteristic dose-response fashion to increasing concentrations of bone marrow colony stimulating factor. Patients with acute leukemia at presentation or relapse (even early relapse) have marrow cells which have colony stimulating factor dose-response curves which are blunted compared to those of normals. Some type of inhibition of normal marrow cell proliferation is, thus, suggested by the presence of leukemia cell clones. This work is designed to identify and characterize this inhibition using an in vitro assay of bone marrow cell growth. An assay of residual leukemia cells in marrow aliquots derived from these studies has been developed and has obvious importance in the management of patients with acute leukemia.

BIBLIOGRAPHIC REFERENCES: Fay, Joseph W., et al.: Leukapheresis Therapy of Leukemia Reticuloendotheliosis (Hairy Cell Leukemia), Blood 54: 747-749, 1979. Hanker, J.S., Ambrose, W.W., Fay, J.W., et al.: Facilitated Light Microscopic Cytochemical Diagnosis of Acute Myelogenous Leukemia, Cancer Research 39: 1635-1639, 1979.

50. IMPROVEMENT OF A BIOASSAY FOR HUMAN TUMOR STEM CELLS

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The objective of this program is to improve the reliability of a recently developed bioassay for human tumor stem cells. This assay is currently being used to study the biological properties of human tumor cells and to predict the efficacy of cancer chemotherapeutic agents. Currently, the low cloning efficiencies of many tumor cells in this system preclude the widespread clinical application of this assay. Therefore, this study will explore improvements in growth conditions for tumor cells. Media conditioned by long-term cell lines will be used to stimulate the growth of primary tumor cells in soft agar. The histochemical, immunological, and cytogenetic characteristics of the tumor cells grown under the new conditions will be described. Initial studies will biochemically characterize stimulators found in conditioned media. The tumor stem cell assay has proven useful both in identifying promising new anticancer agents and for predicting an individual patient's sensitivities to anticancer drugs. The standardization of this bioassay could lead to its use as a predictive tool in cancer chemotherapy and anticancer drug screens.

51. CHARACTERIZATION OF FIBROBLAST AND HEMOPOIETIC CELL LINES TRANSFORMED BY FBJ OSTEOSARCOMA VIRUS

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Unlike most of the mammalian type C sarcoma-inducing retroviruses, FBJ virus was isolated from a spontaneous tumor. The virus is a complex of a non-pathogenic murine leukemia virus (MuLV) and an osteosarcoma-inducing murine sarcoma virus (MSV). Invfection of bone marrow cultures led to the establishment of an early myeloid cell line which induced a chronic myelomonocytic leukemia upon inoculation into syngeneic mice in vivo (see Report 112, and ICRF Scientific Report 1978 - report 117). One cell line, called 426C, originally released both MuLV and MSV; however, it subsequently ceased virus production. Using antisera prepared against disrupted MuLV, proteins can be specifically precipitated from these cells. These proteins are currently being studied by tryptic peptide mapping to determine if they are related to virion proteins. The 426C cell line is being characterized further by biological and immunological criteria. The MuLV component of FBJ virus complex has been cloned by end-point dilution in order to study its biological and biochemical properties. In addition, we plan to study the MSV component by infection of rat and mouse fibroblasts at low multiplicity and selecting for transformed cells by growth in soft agar medium. Colonies will be screened for MuLV production and non-producing clones will be examined for the presence of virus-related proteins, with particular emphasis placed on the detection of transformation-related proteins. Further attempts are being made to establish additional hemopoietic cell lines by infection of bone marrow cultures with the cloned MuLV and the MuLV/MSVFBJ complex. In this way we hope to elucidate the role of each viral component in the transformation of hemopoietic cells.

52. HEMOPOIETIC CELLULAR INTERACTION AND LITHIUM

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The present project is designed to study the regulation of hemopoiesis and in particular characterize interaction between stromal cells, hemopoietic stem cells and other possible intermediary cell types. We plan to utilize clonal stem cell assays, Dexter supernatant stem cell cultures, a number of cell separative techniques including fluorescence activated cell sorting, velocity sedimentation, density centrifugation and adherence separation along with the use of lithium as a probe to evaluate how various cell types interact in the control of hemopoietic stem cell proliferation and differentiation. We also plan to utilize short-term well-defined cultures of endothelium, smooth muscle and fibroblasts to attempt to evaluate their role in the maintenance of hemopoiesis in vitro. In addition we will study genetically anemic SL/SL D and W/Wv mice to attempt to define the defective cellular types and manners in which these types may be manipulated to restore normal hemopoiesis in these animals. Lastly, an attempt will be made to apply these studies to human hemopoiesis and to establish long-term human stromal stem cell maintenance cultures.

53. MYELOID CELL CULTURE

Alsaker, R. D., University of Minnesota, St. Paul Campus, School of Veterinary Medicine, Dept. of Pathobiology, *256 Veterinary Science, St. Paul, Minnesota*, 55101, United States of America

OBJECTIVE: Establish workable culture system for feline myeloid cells. Examine in vitro myeloid growth in feline leukemia virus infested cats. Examine in vitro myeloid growth in cats with drug induced neutropenia.

APPROACH: Establish culture system by modification of existing techniques. Detect feline leukemia virus infected cats by immunofluorescence and culture of their bone marrow cells. Give cats drugs with known depressive effects on myelopoiesis (e.g. chloramphenicol); examine effects on stem cell in culture system.

B. LYMPHOID CELLS

54. THE CHARACTERIZATION OF A5A IDIOTYPIC SUPPRESSOR CELLS

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The objective of this proposal is to characterize the T cell receptor on the A5A idiotypic suppressor T cell described by Dr. Klaus Eichmann.

The proposed study will attempt to establish cultures of A5A plus enriched suppressor T cells by induction of suppressor cells with guinea pig IgG2 anti-A5A-idiotype serum. Suppressor cells will be enriched by using plates or columns coated with anti-idiotype serum as an immune absorbant. Long-term cultures of these cells will be established using cell free supernatants from Con A stimulated spleen cell cultures as a supportive medium. Cloning of these established suppressor cell cultures will be attempted.

The cultured suppressor populations and clones will be functionally analyzed as to enrichment of suppressor activity, cell surface markers, and the specificity of these cells for antigen and the A5A idiotype using in vitro microcultures and PFC assays.

Finally selected suppressor cell lines will be characterized as to the nature of their cell surface receptor using radiolabeling and immunoprecipitation techniques to isolate the receptor, and chromatographic techniques to study its properties.

55. STUDY OF THE METABOLIC AND STRUCTURAL CHARACTERISTICS OF THE HETEROGENEOUS NUCLEAR RNA IN HUMAN LEUKEMIC CELLS

Torelli, U. L., Torelli, G., Narni, F., Ferrari, S., Donelli, A., Franchini, G., Universita degli Studi di Modena, Inst. of Medical Clinics, *Policlinico, Via del Pozzo, 41100 Modena, Italy*

OBJECTIVE: The investigation is aimed to explore the existence of structural and metabolic differences between heterogeneous nuclear RNAs (hnRNA) extracted from normal and leukemic cells.

APPROACH: Experiments were carried out to study the kinetics of hybridization in vitro to human DNA of hnRNA extracted from three types of cells: normal human promyelocytes, normal human lymphoblasts and leukemic human promyelocytes of the HL-60 cell line. In the labeled hnRNA from the two normal cell types, at least 15% of the sequences are complementary to reiterated sequences, and at least 25% are complementary to single copy sequences. In the labeled hnRNA from HL-60 cells, the least squares computer analysis indicates that at least 30% of the sequences are complementary to reiterated DNA sequences. Furthermore, the analysis did not suggest the presence of a class of sequences definitely corresponding to the single-copy sequences observed in hnRNA from normal cells. It is concluded that the increased repetitiousness of the heterogeneous nuclear RNA in the HL-60 cells is a leukemia-related characteristic of the cells.

PROGRESS: Several lines of research have been suggested by the findings reported above. Priority has been given to the direct determination of the complexity of the hnRNA from normal and leukemic cells. Such determination is now in progress.

56. CHARACTERIZATION OF UNIQUE LYMPHOBLASTOID CELL LINES

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First, I propose to establish long-term lymphoblastoid cell lines (LCLs) from individuals with genetic disorders characterized by predisposition to cancer and/or hereditary tumors. This will be accomplished by transforming isolated blood leukocytes in vitro from affected individuals, using high titer Epstein-Barr virus (EBV). Secondly, I propose to study the in vitro sensitivity of these LCLs, as well as normal LCLs, to different chemical and physical mutagens. On a molecular level the amounts and types of DNA repair synthesis induced following exposure to DNA damaging agents will be quantitated for LCLs established from each disorder. In addition to these viability and repair studies, mutation frequencies for three different genetic markers will be examined in these LCLs following treatment with specific mutagenic agents.

These studies will help identify those DNA damaging agents for which these individuals show increased sensitivity and which may increase their risk of developing cancer. The expected increased sensitivities of these same LCLs may also allow the identificaton of compounds that, following exposure, increase the risk of cancer for the general population as well. The proposed combined molecular and biological studies on repair replication and mutation frequencies will help us understand more fully the relationships between normal repair synthesis, defective repair synthesis and somatic cell mutation in mammalian cells.

BIBLIOGRAPHIC REFERENCES: E.E. Henderson and R. Ribecky. Transformation of Human Leukocytes with Epstein-Barr Virus after Cellular Exposure to Chemical or Physical Mutagens. J. Natl. Cancer Inst. 64:33-39, 1980. Levitt, M., D. Shungu, and E. Henderson. Colony Morphology, Cell Growth, and Viral Radiosensitivity as Parameters for Detecting Strain Differences in Epstein-Barr Virus (EBV). Int. J. Cancer 26:509-515, 1980.

57. GENETIC AND BIOCHEMICAL ANALYSIS OF HUMAN LYMPHOID CELL SURFACE ANTIGENS

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The summary for this project is under entry no. 26.

58. PROPAGATION OF THYMUS-DERIVED LYMPHOCYTE LINES

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The overall goal of this project is an in-depth analysis of the thymus derived lymphocyte and its products. The basic approach is to use continuously propagable T cell lines which express specificity for individual antigens. Highly purified, functionally characterized, antigen sensitized T cells will be fused with extensively analyzed, cloned T cell lymphomas to make T cell hybrids. Lymphoma parents will be chosen which most nearly approximate the differentiated state of the primary T cell. In other experiments purified T cells will be treated with antigen and T cell growth factor. Antigen-specific continuous T cell lines will be used as source material to analyze the parameters which regulate T cell recognition of antigen. T cell lines will be used as source material for molecular analysis of lymphokines and T cell receptors by SDS-polyacrylamide gel electrophoresis, column chromatography, and two-dimensional gel electrophoresis.

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59. TUMOR-ASSOCIATED ANTIGENS AND NEOANTIGENS ON HUMAN MALIGNANT LYMPHOID CELLS AND LYMPHOBLASTOID CELL LINES

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This project is aimed at the study of tumor-associated cell surface antigens of human lymphoid malignancies and neoantigen expression of cultured lymphoblastoid cell lines. For this purpose, xenogeneic monospecific antibodies will be generated in vitro using the mouse spleen fragment culture system, as developed by Klinman. Fragments will be isolated which produce homogeneous, monoclonal antibodies recognizing specifically human malignant lymphoid cells or cultured lymphoblas-

toid cells, but not normal lymphoid cells. The objective will be to determine the number of tumor-associated antigens and neoantigens on malignant lymphoid cells and cultured lymphoblastoid cells, respectively, and the degree to which these antigens are shared by related tumor cells and cell lines.

In addition, because antibody producing fragments have a limited life span in vitro, we will attempt to expand the specific antibody producing clones in such fragments by fusing them to mouse myeloma tissue culture cell lines, as described by Kohler and Milstein. In this way, hybrid cell lines can be grown to produce monospecific anti-tumor antibody. These antibody producing hybrid cell lines can be propagated indefinitely and lead to the large-scale production of specific antibody. Such monospecific anti-tumor antibodies produced in vitro may be applicable to immunodiagnosis and immunotherapy.

60. IDENTIFICATION OF IMMUNOLOGICAL MARKERS IN LEUKEMIC CELLS - CRYOPRESERVATION AND LONG-TERM CULTURES

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OBJECTIVES: Identifcation of different types of leukemias using specific immune markers. Preparation of specific ALO antisera to permit identification of other leukemias of the same type. Viable conservation by freezing long-term cultures, as a method of obtaining a source of antisera for later immunotherapeutic treatment of the same patient, or a cross-reaction with similar patients.

APPROACH: Peripheral blood samples are taken from patients with leukemia (initially only from patients with acute lymphoblastic leukemia). Using the ficoll-Hypaque technique, separate mononuclei and separate phagocytes from them to obtain only lymphocytes. Trypan blue is needed for viability testing. Rabbits and goats are injected; antiserum absorption; identification of the specific antigen obtained by cytotoxicity.

The study will be repeated with patients in remission. Cryopreservation will be effected in RPMI 1640 and DMSO in liquid nitrogen container. Cultures of lymphoid cells will follow the method of MacCullock and Till.

Techniques and preliminary trials will be standardized.

61. CULTIVATION OF HUMAN T-LYMPHOCYTES IN AGAR - AN IMMUNOLOGICAL PARAMETER

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OBJECTIVES: A) Functional characterization of the TL-CFU grown in agar. B) Comparison of the results of the TL-CFU characterization with the results of other immunological function tests such as stimulation of lymphocytes in liquid culture with PHA, Con-A and PWM.

APPROACH: A) Determine whether TL-CFU grown in agar have receptors for IgG or IgM and if they can help the B-lymphocytes to differentiate to plasma cells. B) We take PBL from patients with breast cancer and control persons and measure the capacity to form colonies in agar after stimulation with PHA; these results are compared with the capacity for thymidine uptake after simulation with PHA, Con-A or PWM.

PROGRESS: T-lymphocytes grown in agar have been found to help the B-lymphocytes to differentiate to plasma cells if stimulated with PWM.

62. CELLULAR IMMUNITY TO TUMORS AND HERPES INFECTIONS

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The research proposed for the remaining four months of this grant is directed at continuing our studies aimed at generating cytotoxic lymphocytes in vitro, capable of lysing autologous human leukemia and lymphoma cells. Attempts will be made to generate cytotoxic T lymphocytes (CTLs) in mixed leukocyte cultures (MLCs) by culturing remission T cells with a) x-irradiated autologous leukemia cells and x-irradiated allogeneic stimulating cells in 'three-cell' protocols or with b) x-irradiated lymphocytes pooled from 20 unrelated normal individuals. The addition to these MLCs of T cell growth factor (TCGF), highly purified human fibroblast interferon (HFIF) or polyribonucleotide inducers of interferon (IF) may augment the generation of CTLs to autologous malignant cells since these agents markedly enhance the generation of CTLs to alloantigens. A main goal of this research will be to generate large numbers of CTLs cytotoxic for autologous malignant cells; thus, continuous cultures of CTLs, cytotoxic for autologous malignant cells, will be established in TCGF. Further, we propose to begin to isolate clones of CTLs, cytotoxic for autologous malignant cells, with which to determine whether malignant cells from different patients express common target antigens for CTLs.

63. A CULTURE METHOD FOR QUANTITATING PROLIFERATION AND DIFFERENTIATION OF HUMAN NEUTROPHILIC GRANULOCYTES

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We have developed a liquid culture method and assay techniques which have permitted more precise study of proliferation and differentiation of mouse granulocytes. An important feature of the culture method is its adaptability to both the microscopic methods (clone counting and sizing, 3HTdR autoradiography, immunocytofluorescence of stage specific proteins) as well as expedient quantitative biochemical techniques (3HTdR incorporation into DNA by scintillation counting, DNA quantitation, radioimmunoassays of specific proteins). Using this methodology we have demonstrated that CSA in high concentration enhances proliferation in clones of neutrophilic granulocytes while delaying differentiation at the promyelocyte-myelocyte stage.

The purpose of this proposal is to adapt for use with human cells the culture techniques and assays of proliferation and differentiation which we have found useful in the mouse studies. Normal and leukemic human granulocytic cells are not as co-adhesive as normal immature mouse granulocytes and lose their clonal orientation in liquid culture. We therefore have found it necessary to culture the human cells in semi-solid rather than liquid media. Techniques are being developed to obtain suitable preparations of the entire cultures for the microscopic assays (e.g. immunocytofluorescence), and to retrieve total culture cells from the semi-solid media for the immunobiochemical assays.

Once the developmental work on the culture method is complete, we propose to validate this method as an assay of clonal granulopoiesis, and begin to apply the techniques in quantitating the effect of various factors on the time course on in vitro neutrophil differentiation. The possibility that these methodologies may permit distinguishing normal from leukemic clonogenic cells will also be explored.

64. STUDIES ON IMPAIRED LYMPHOCYTE FUNCTION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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OBJECTIVE: To explore the mechanism of the differentiation block of B lymphocytes, preventing those from becoming plasma cells, in chronic lymphocytic leukemia, by means of cell culture techniques, and to devise means of overcoming this block. In addition, we wish to use immunological means to increase the number and function of any remaining 'normal' B lymphocyte clones. The eventual object is to find out how to stimulate immunoglobulin (antibody) synthesis in vivo, since low immunoglobulin levels are a common cause of recurrent infections in this disease.

APPROACH: A modification of the Jerne technique in vitro will be employed. This involves culturing B lymphocytes and stimulating them to become plasma cells (antibody-forming cells), which are assayed by plaque formation (hemolysis) of erythrocytes in agar. Various types of stimulants will be tried. Cell separation techniques will be used to test the effect of adding or removing different lymphocyte populations in normal and leukemic cell cultures.

65. CLONOGENIC, CYTOKINETIC AND DIFFERENTIATIVE PROPERTIES OF CELL POPULATIONS IN HUMAN LEUKEMIAS AND LYMPHOMAS (ABBREV)

Gavosto, F., Gallo, E., Pagliardi, G. L., Aglietta, M., Novarino, A., Matera, L., Sanavio, F., Universita degli Studi di Torino, Medical & Surgery Faculty, I Cattedra Patologia Medica, *Corso Polonia 14, 10126 Turin, Italy*

The aim of our study is a better knowledge of the mechanisms of leukemic proliferation and a characterization of factors of prognostic relevance to lympho- and myeloproliferative disorders. Moreover, the technique for cryopreservation of hemopoietic cells, as a first step towards autologous bone marrow transplantation, will be standardized. Our recent studies on leukemic cell kinetics produced the following results: 1) In human AML, high doses of Ara-C, although producing cell kinetic perturbations, did not induce a synchronous wave of recruitment of leukemic resting cells; 2) in BC of CML a different cell kinetic pattern for lymphoblastic and myeloblastic BC has been observed; 3) in AL blasts with membrane receptors demonstrate a higher proliferative activity than blasts without receptors.

The studies on the prognostic value of circulating IC are at an advanced stage: 1) In AL and non-Hodgkin's lymphomas the presence of IC correlates with the amount of neoplastic tissue; 2) IC, absent in CML, appear after the evolution to BC.

The effect of lithium on normal and leukemic CFU-GM has been investigated. Preliminary results indicate that while normal CFU-GM are slightly stimulated by lithium, leukemic CFU-GM are insensitive.

We obtained and stabilized a cell line from a patient with erythroleukemia. There is preliminary evidence that, in the presence of hemin, hemoglobin is synthesized.

66. DEVELOPMENT OF A METHOD FOR THE ROUTINE PRODUCTION OF LARGE VOLUMES OF LYMPHOID LINES

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We are currently growing lymphoid cell lines in a 10-liter vessel that has been adapted to enable up to 5 liters of suspension to be decanted with the replacement of fresh medium, thus enabling their continuing growth in situ. We propose to develop this system to enable us to culture continuously 10 by 10 liter vessels.

67. COLONY FORMING UNITS IN CULTURE OF CHILDHOOD ACUTE LEUKEMIA

de Koning, J., van 't Veer Korthof, E., van Weel Sipman, M. H., Academisch Ziekenhuis Leyden, Dept. of Pediatrics, *Rijnsburgerweg 10, 2333 AA Leyden, Netherlands*

Colony forming units in culture (CFUC) technique is used for the diagnosis of acute lymphoid leukemia versus other forms of leukemia in childhood. An attempt is made to anticipate recurrence of this leukemia earlier on the basis of growth patterns in the CFUC. Results achieved with this method are compared with the results of conventional diagnostic tools (cytomorphology etc.). Since the start of protocol 220, CFUC technique has been used in examination of bone marrow and blood of 117 patients with various hematological diseases. Twenty-one of the patients suffered from acute lymphoid leukemia, 4 from acute myeloid leukemia and 2 from chronic myeloid leukemia.

68. IN VITRO STEROID SENSITIVITY OF NEOPLASTIC HUMAN LYMPHOCYTES

Duval, D. P., Homo, F. G., Inst. National de la Sante et de la Recherche Medicale, Physiology & Pharmacology Unit, *Hopital Necker, 161 Rue de Sevres, 75015 Paris, France*

OBJECTIVE: To develop an in vitro assay for determination of steroid sensitivity of neoplastic human lymphocytes.

APPROACH: Lymphocytes are isolated from the blood of patients with acute lymphoblastic leukemia by centrifugation on ficoll-Hypaque. Using cell suspensions, the binding of 3H-dexamethasone in the presence and absence of excess cold DM is measured in order to determine the level of glucocorticoid receptors; secondly, the inhibition of 3H-uridine and 3H-thymidine incorporation into nucleic acids is determined following 24 hours incubation at 37 degrees C in vitro in the presence of various concentrations of steroids and vincristine; thirdly, the immunologic markers and the proportion of cells in the S phase of the cell cycle (analysed by pulsed cytophotometry) are determined in parallel. When possible, these parameters are determined in subpopulations isolated by centrifugation on bovine serum albumin gradient.

PROGRESS: In 25 patients with acute leukemia there is no correlation between the level of glucocorticoid receptors and the in vitro steroid induced inhibition of nucleoside incorporations.

69. CYTOPATHOLOGICAL STUDY ON MALIGNANT LYMPHOMA USING MICROPHOTOMETRY AND CELL CULTURE

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OBJECTIVE: To delineate the pathological and cytopathological characteristics of various kinds of malignant lymphomas as well as to differentiate and evaluate their malignity, cytomorphological and cytobiological features were extracted and the results were correlated with the histological classification of lymphoma. Follicular lymphomas are often difficult to differentiate from reactive hyperplasia and differentiation between the benign and malignant forms is also difficult. Reticulum cell sarcoma is frequently difficult to differentiate from lymphosarcoma. To find a solution to these problems, research and experimental studies are carried out as described below.

APPROACH: In addition to the routine histology sections, imprint smears were made from the cut surface of the biopsied and/or autopsied lymph nodes or lymphoid tissue. They were stained with Feulgen reaction; besides various routine stainings Olympus MMSP (microphotometer) was provided to measure the nuclear DNA content of the Feulgen-stained materials. Electron-microscopical observation and the method for T- and B-cell marker detection were applied to various kinds of lymphomas.

PROGRESS: Comparing the histograms of the nuclear DNA content of various kinds of lymphomas, characteristic features were disclosed as follows; mature lymphosarcoma and follicular lymphomas show a pattern close to the normal range, but premature and pleomorphic cell types show wide distribution in ploidy on the histogram. Hodgkin's cells and/or Reed-Sternberg giant cells often show a pattern resembling that of malignant mode. Data on various lymphomas are being accumulated.

70. FUNCTIONAL ANALYSIS AND REGULATION OF LEUKEMIC CELLS

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In vitro leukemic cell lines now exist which express a variety of type-specific functions characteristic of normal hematopoietic cells, including induction of differentiated properties by humoral regulators. The cell lines themselves produce regulatory molecules that mediate specific stimulatory or inhibitory actions on hematopoiesis, as well as on the functions of normal mature end cells. The complexity of hematopoietic regulation requires a multiparameter analysis and purified cell preparations in order to understand the functional properties of leukemic cells. The objectives of this proposal are to establish cell lines of human and mouse leukemias, to define the phenotypes of leukemic cells, to study their retention of responsiveness to regulatory control and activation of functions, to assay for the production of molecules stimulating or inhibiting normal cell functions, and to determine differential sensitivity of leukemic cells to toxicity or growth inhibition by cell type-specific drugs. Primary cultures and established lines of leukemic cells will be assayed for production of lysosomal enzymes, myeloid colony-stimulating activity, lymphocyte activation factors and prostaglandins; for T lymphocyte-specific terminal transferase; for immunoglobulin, surface receptors and antigens characteristic of a blood cell lineage; for a soluble inhibitor of normal hematopoiesis found in leukemic patients; and for nonspecific and antibody-dependent phagocytosis and cell-mediated cytotoxicity. The response of these cells to macrophage and lymphocyte-activating substances will be tested for inhibition of cell proliferation in

parallel with induction of differentiated surface markers and functional properties. A culture system supporting continuous marrow stem cell proliferation will also be used in which spontaneous and viral leukemogenesis has been demonstrated. Certain chemotherapeutic drugs will be tested in vitro for subtype-specific toxicity. The emphasis of the program will be directed at improvements in diagnosis, defining regulatory derangements in leukemia, and determining the interactions between normal and leukemic cells.

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71. STUDIES OF MITOSIS IN NORMAL AND NEOPLASTIC CELLS

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The objective of the present proposal is to elucidate the ultrastructural and functional features of the mitotic apparatus (MA) in dividing cells. Experiments are designed to gain further insight into the structure and assembly of the MA and its function in the control of chromosome segregation in normal and neoplastic cells. The materials to be investigated include cultured mammalian fibroblasts, HeLa cells, human lymphocytes from normal and leukemic patients, avian fibroblasts, insect spermatocytes, and various mammalian cell hybrids. The cell type to be used in each experiment will be selected to facilitate ease and simplicity of the experimental design.

The areas to be investigated include (a) further studies of the ultrastructure and function of the kinetochore, (b) further studies of the centriole and its role in the assembly of the MA and chromosome segregation, (c) relationship between the components of the nuclear envelope and the kinetochore, (d) further studies of the effects of drugs and physical agents on the MA, and (e) mitotic events in mammalian cell hybrids.

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72. TRANSCRIPTIONAL CONTROL OF GLOBIN GENES IN FRIEND LEUKEMIA CELLS

Nose, K., Okamoto, H., Itoh, N., Toyama Medical & Pham. Univ., School of Medicine, Dept. of Biochemistry, *Sugitani, Toyama, Japan*

OBJECTIVE: Elucidation of control mechanisms in the transcription of globin genes.

APPROACH: Nuclei or chromatin is isolated from noninduced and induced Friend leukemia cells and transcribed either with endogenous RNA polymerase or purified polymerase II from wheat germ. Contents of globin mRNA in the transcripts are titrated with globin cDNA. Various noninducible variant cells are used at the same time, and nucleic acid proteins in these variants and in wild-type cells are compared.

An attempt is also made to isolate 'transcriptionally active' chromatin fragments from wild-type and mutant cells with DNase I and DNase II.

73. STUDIES ON CYTOTOXIC T LYMPHOCYTES

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OBJECTIVES: To elucidate the mechanisms involved in the production of both cytotoxic T lymphocytes (CL) and their precursors (CLP).

APPROACH: Using quantitative in vitro culture techniques and inbred mice, one can measure relative CL number, CLP frequency, CL per CLP, helper cell activity and suppressor cell activity. Using this underlying technology, two particular approaches are planned: 1) Using a flow cytometer-cell sorter and the fluorescent vital stain H33342, one can detect and sort specific CLP within 12 hrs of culture initiation. This enables one to decouple the two signals required for CLP activation and should enable a more complete dissection of the cellular requirements for CL production than previously possible. 2) T cell colonies can be grown from normal mouse bone marrow and from the spleens of normal and athymic nude mice. The colonies from each tissue contain a variety of functional cell types (CLP, helper, suppressor) characteristic of the tissue. The Lyt and Ia antigen distributions within individual colonies will be measured by flow cytometry and correlated with function. Colonies containing CLP contain CLP of several specificities and it will be investigated whether diversification is occurring within the colony.

74. EXPRESSION AND FUNCTION OF DIFFERENTIATION ANTIGENS ON MURINE LYMPHOCYTES AND LEUKEMIA CELL LINES

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OBJECTIVES: Expression and function of differentiation antigens on murine lymphocytes and leukemia cell lines.

APPROACH: Preparation of conventional and hybridoma antibodies to lymphoid cells, their characterization by physicochemical and immunochemical techniques, and their use to define functions of cell subpopulations and cell membrane constituents.

PROGRESS: A hybridoma antibody has been prepared which reacts with approximately 50% of B-lymphocytes, and about 40% of bone marrow cells. Another hybridoma antibody reacts with a largely non-overlapping population of B-lymphocytes and bone marrow cells. Functional characterization of these subpopulations is in progress. Subsets of B-lymphocytes, as defined by surface isotypes, have been prepared, and their capacity to respond to T-independent and T-dependent antigens, in the presence and absence of anti-IgD antibody, has been examined. An interesting finding was that IgD-positive cells responded to both types of antigen, but only in the T-dependent case did anti-IgD inhibit the response.

75. H-2 ANTIGEN EXPRESSION IN CULTURED MOUSE LEUKEMIA CELLS

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The aim of the proposed research is to study the regulation of expression of the H-2 antigens. The approach will be to isolate, from heterozygous mouse leukemia cells in culture, variants that have undergone stable heritable alterations in the expression of the H-2 antigens and to use these variants to investigate the normal controls of H-2 antigen expression. Variants will be isolated by two in vitro techniques. One of these, anti-H-2 antibodies in the presence of complement, has been used successfully in the past two years to isolate several interesting variants. More variants will be sought using this method. In addition, H-2 directed cytotoxic T-cells will also be used as selective agents.

Once isolated, the variants will be studied by genetic techniques such as karyotyping and somatic cell hybridization; biochemical procedures such as gel-electrophoresis and, in collaboration with Dr. S.G. Nathenson, peptide mapping, where warranted; and by immunological methods such as radioimmunoassay, serological responses on inoculation into the syngeneic animal, and interactions with T-lymphocytes, immunized against wild type antigens.

The rates of emergence of these variants will be determined by the fluctuation analysis. This experiment will also be used to determine whether the variants preexist the selection, or are in some way induced by the selective agents. The effect of various mutagens on the rates of emergence will be studied.

Recently, much attention has been focused on the role of the H-2 antigens in the immune response to viral antigens. The H-2 variants and the wild type cells form an isogenic system where this role of the H-2 antigens can be studied. The immune responses of syngeneic animals to the Friend virus associated

antigens will be investigated. Some preliminary experiments have suggested that certain combinations of H-2 antigens may interfere with or augment the T-cell response to the antigens associated with the Friend virus.

C. NEUROBLASTOMA AND OTHER BRAIN CELLS

76. DETAILED CELL KINETIC ANALYSES OF HUMAN NEUROBLASTOMA

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The overall goal of this project is to increase our basic knowledge of the biology of cell replication in human neuroblastoma. Two models will be studied: the first consists of several human neuroblastoma lines in vitro and the second consists of these neuroblastoma lines grown in solid tumor form in the immunodeficient nude mouse. These tumors will be examined for synchrony, diurnal variations, and changes in proliferative characteristics with increasing size. The classic cell kinetics measurements, such as labelling and mitotic indices, and fraction-labelled mitoses curves, will be performed to determine baseline cell cycle times and parameters. Several newer techniques will be used also. First, flow cytofluorometry will be used to study overall changes in DNA distributions wthin the tumors. Combined tritiated thymidine autoradiography and DNA cytofluorometry will be used to provide independent measurements of DNA synthesis status and cell cycle position to study the kinetic characteristics of proliferating and non-proliferating cells. These studies will provide a detailed analysis of the cell kinetics of untreated neuroblastoma in order to provide a baseline with which treatment can be rationally formulated and against which therapeutic efforts can be objectively compared.

BIBLIOGRAPHIC REFERENCES: Neely, J.E.: Constancy of Cell Kinetic Parameters in a Human Neuroblastoma Line in the Nude Mouse, in Recent Advances in Neuroblastoma Research (A.E. Evans, ed.), 1980, in press.

77. STUDY OF MALIGNANCY IN HUMAN TUMOR CELL LINES

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Cell lines from two presumed genetic human tumors - neuroblastoma and retinoblastoma - will be used to study several aspects of oncogenesis. Chromosome analysis of the tumors and cell lines will be performed to determine if non-random chromosomal changes are characteristic of each tumor type. The functional significance of two chromosome abnormalities already described in these tumors - the Homogeneously Staining Region and double minute chromosomes - will also be analyzed. Somatic cell hybridization (involving the fusion of the tumor cells with other malignant and non-malignant cells of the same or different species) and the technique of gene mapping will be used to study the pattern of inheritance of the malignant phenotype in these tumors and to assign the genes involved in the regulation of malignancy to individual chromosomes.

78. CYTOGENETIC AND TRANSPLANTATION STUDIES ON NEUROBLASTOMA CELL LINES (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

79. AN INVESTIGATION OF THE INTERRELATIONSHIP OF BRAIN TUMOUR DIFFERENTIATION AND GANGLIOSIDE COMPOSITION

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OBJECTIVE: The objective is to examine how far the ganglioside composition of brain tumour cells may reflect the degree of differentiation of the cells and whether or not any alterations also indicate changes of protein composition in the plasma membrane, or merely the failure of glycosylation.

APPROACH: Human brain tumour samples and cultured cell lines are to be analyzed for their ganglioside composition. The relationship between composition and histopathological classification will be examined, in particular with respect to the degree of differentiation of the tumour. Where possible cultured cells will be examined by immunofluorescence with specific antiganglioside sera.

The synthesis of ganglioside by tumour cells in culture will be examined, particularly that of the glial cells, and compared to their synthesis by normal cells. Particular attention will be paid to possible metabolic compartmentation in the glia and to the presence of exo-transglycosylases. Using specific antiganglioside antibodies, affinity methods will be used to look for membrane complexes containing gangliosides. If such complexes are found, then the protein and lipid constituents of such complexes will be partially characterized. The occurrence of other components will then be sought in tumour cells showing a depleted or abnormal ganglioside composition.

80. CONTROL OF GLIAL FIBRILLARY ACIDIC PROTEIN FORMATION IN HUMAN BRAIN TUMOR (GLIOMA) CELLS

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This proposal is designed to study glial fibrillary acidic protein (GFAP) in human glioma cells, to demonstrate factors that effect the formation of the protein, to relate GFAP formation to growth characteristics of glioma cells in culture, and to examine whether any of these characteristics relate to the clinical course or therapeutic response of the donor. GFAP is a specific marker for astrocytes and is believed to be associated with the 9 nm cytoplasmic filaments. The protein will be localized by the immunoperoxidase method and quantitative determinations done by rocket immunoelectrophoresis. Six factors, known to affect glioma cell growth or differentiation, will be studied for their putative effects on GFAP formation. The factors to be studied include polyamine metabolism, low serum concentration in culture media, dimethylsulfoxide, butyric acid, cyclic 3'5 AMP, and amethopterin.

81. HUMAN GLIOMA HETEROGENEITY AND CHEMOTHERAPY

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Evidence exists that human brain tumors are composed of heterogeneous cell populations that account for their pleomorphism, biological change toward increased malignancy over time, and the occurrence of mixed gliomas and gliosarcomas. Such heterogeneity may also be responsible for the relatively poor results achieved in the chemotherapy of patients harboring malignant gliomas. To date, chemotherapy has usually been limited to single agents and this form of treatment may permit overgrowth of resistant cell lines. In our laboratory, different human malignant brain tumors growing in athymic nude mouse brains have displayed different chemosensitivities to standard agents, suggesting variability among gliomas and perhaps among different cell lines within each glioma. We propose to clone out these heterogeneous cell populations in tissue culture from gliomas obtained from patients undergoing resections of their tumors. The clonal lines will be characterized by morphology (light and electron microscopy), biochemical markers (S100 protein, glial fibrillary acidic protein, glycerol-3-phosphate dehydrogenase, cyclic nucleotide phosphohydrolase), karyology, growth kinetics, chemosensitivity, and cell surface markers. The parent lines and clones will be inoculated intracerebrally and subcutaneously into athymic nude mice to determine in vivo morphology, growth kinetics and chemosensitivity. The clone lines will be cocultivated to determine if phenotypic expression for drug sensitivity is altered and, if so, whether by intercellular exchange of diffusible substances and/or by cell-cell communication. Finally, utilizing the chemosensitivity profiles of the individual clones and information from cocultivation, new chemotherapy combinations will be designed and tested on the parent lines in vitro and in vivo. An attempt will be made to correlate chemosensitivities with other characteristics to find

early identifiable markers in order to suggest chemotherapy regimes for the patient as early as possible.

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82. CELL SURFACE ANTIGENS OF GLIAL BRAIN TUMOR CELLS

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Surface antigens of human glial brain tumor cells and VM murine spontaneous anaplastic astrocytoma cells were investigated by binding (indirect membrane immunofluorescence) and complement-dependent cytotoxic antibody (14C-nicotinamide release and dye-exclusion) assays. Antisera from nonhuman primates immunized with glioblastoma multiforme tissue or cells from permanent human glial brain tumor cell lines have been extensively absorbed to analyze which human glioma cell surface antigens may be species-, tissue-, or glial tumor-specific; oncofetal; or cross-reactive with non-glial tumor cell antigens. We have established an inbred colony of VM/dk mice, an in vivo transplantable VM astrocytoma line, and 3 VM astrocytoma cell lines. We will extend the initial finding of human glial tumor-associated antigenicity with additional primate antisera now in production and an expanded panel of neoplastic and non-neoplastic adult and fetal glial and non-glial human cell lines. Syn-, allo-, and xeno-geneic antisera raised against the VM astrocytoma will be analyzed for activity against murine oncornaviruses; antisera suitably absorbed will be analyzed for astrocytoma-associated antibody activity. In both systems we will extract from culture supernatants or cell membrane preparations antigenically active moieties by monitoring the process in inhibition assays with absorbed specific antisera. Radioimmunoprecipitation of labeled reactive antigenic moieties will be followed by polyacrylamide gel electrophoresis. Partial purification of solubilized antigens will be performed by gel filtration, ion exchange chromatography, and other gentle methods of protein separation. Specific antisera and solubilized antigens will be used to determine if body fluids of patients or cancerous mice contain glioma-distinctive antigens, measurement of which may serve diagnostic, disease process-monitoring, or immunotherapeutic purposes.

BIBLIOGRAPHIC REFERENCES: Laerum, O.D., Bigner, D.D., Rajewsky, M.F., (eds.) Biology of Brain Tumors, International Union Against Cancer Technical Report Series - Volume 30, 209 pp. Geneva, 1978. Bigner, D.D.: Chapter IV, Role of Viruses in the Causation of Neural Neoplasia, pp. 85-99, in International Union Against Cancer Technical Report Series - Volume 30, 209 pp. Geneva, 1978.

83. BIOLOGICAL PROPERTIES OF FETAL BRAIN CELLS DURING MALIGNANT TRANSFORMATION IN CELL CULTURE

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OBJECTIVES: a) Investigation of phenotypic properties of fetal rat brain cells during the gradual malignant transformation in cell culture. b) Use of malignant neurogenic cell lines for experimental therapy.

APPROACH: The project was started in Professor M. F. Rajewsky's group in Tubigen and is continued in close cooperation with his institute in Essen, Germany. Pregnant rats, 18th day of gestation, are given a single pulse of ethylnitrosourea, which gives a very high yield of neurogenic tumors in the offspring. Shortly after the in vivo carcinogen pulse, the brains of the fetuses are transferred to cell culture, where they undergo gradual malignant transformation until they are tumorigenic after a period of 200 days. The differentiation of neoplastic and pre-neoplastic cells is studied using different nervous system specific markers. Karyotyping and measurement of cellular DNA content are performed in parallel.

PROGRESS: The experimental system has been reported on earlier and results from detailed electron microscopic analysis of the same sequence of transformation are published in the same journal. Different classes of neural cells which participate in the process of neoplastic transformation have been identified by transmission electron microscopy. Concomitant alterations of cytoskeleton have been investigated in parallel. Expression of different nervous system specific markers is under investigation.

Cytostatic effects of different platinum compounds on such neoplastic cell lines have been investigated under in vivo and in vitro conditions. Combination therapy with cytostatic drugs and hypothermia is in progress.

84. MATURATION FACTORS AND CANCER CELLS

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Glia maturation factor is a protein factor found in the adult brain that has the ability to enhance the phenotypic expression of normal immature astrocytes in culture. In the proposed work, experiments are planned to test the effect of the factor on various neoplastic glial cells. Morphological, biological and chemical parameters will be used to measure the effect. We are trying to determine if the factor blocks the appearance of some of the neoplastic properties of the cells in an otherwise permissive environment. It is hoped that the study will provide some insight into the interaction between the maturation factor and the malignant process.

85. IN VITRO DIFFERENTIATION OF CLONAL CELL LINES FROM MAMMALIAN NEUROTUMORS

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Our overall objective is to study mammalian neurocell differentiation and development using various rat neural cell lines isolated from ethylnitrosourea-induced tumors. Biochemical and cytological characterization of several cell lines clonally isolated from a rat peripheral neurotumor, RT4, has been conducted and their neuronal and glial properties examined. A particularly interesting phenomenon we have found is 'cell-type conversion,' in which a multipotential stem cell line consistently converts to three different cell types in culture and these derivative cell types possess both properties. An interesting feature of this system is that tumorigenicity consistently correlates (couples) with S100 protein production. Both the stem cell line and a derivative cell line without voltage-dependent Na ion-influx but with S100 protein ('glial' type) are tumorigenic, and two other derivative cell lines with voltage-dependent Na ion-influx but without S100 protein ('neuronal' type) are nontumorigenic. This system will be further studied biochemically and cell biologically. Major questions asked concern 1) individual cell analysis of the stem cell line for neuronal and glial properties, 2) segregation patterns of other neuronal and glial properties, and 3) in vivo differentiation of neuronal and glial properties of neurocells in sympathetic ganglia.

D. MAMMARY CELLS

86. CELL HYBRIDIZATION STUDIES ON THE HORMONAL REGULATION OF BREAST TUMOR CELL GROWTH IN CULTURE

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The summary for this project is under entry no. 36.

87. THE INFLUENCE OF COMBINATIONS OF ESTROGENS AND ANDROGENS ON CULTURED MAMMARY CARCINOMA CELLS

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OBJECTIVE: To study the possible interference of androgens and estrogens in mammary cancer cells.

APPROACH: Our studies are focused on the effects of estradiol-17-beta and 5-androstene-3-beta, 17-beta-diol on the MCF-7 cell line and cells cultured from primary mammary tumors. The androgen androstenediol was chosen because of its high relative binding affinity for the estrogen receptor. These

two hormones are applied separately or in combination to investigate their influence on DNA synthesis, growth, estrogen receptor translocation, progesterone receptor synthesis and the cell cycle. Results obtained with the MCF-7 cell line will be used in designing experiments with cultured primary tumors.

PROGRESS: In MCF-7 cells androstenediol is behaving like a weak estrogen (concentration ratio 100:1 with respect to estradiol). Combinations of these hormones do not show any significant interferences between estradiol and androstenediol with regard to the capability of each steroid to stimulate growth of MCF-7 cells. Similar experiments concerning the progesterone receptor synthesis are in progress. Methodically, the uptake of 3H-thymidine was compared to the labeling index in MCF-7 cells (Coosen et al., 1980, submitted to Mol. Cell. Biochem.). Estradiol appears to enhance both parameters to the same extent, but the former only for 24 hours after hormone addition. This correlation may be of use in comparing biochemical and autoradiographic data on DNA synthesis in the case of culturing both primary mammary tumors and mammary cancer cell lines (i.e. MCF-7). This approach is currently being investigated.

88. METABOLISM OF ESTROGENS AND ANDROGENS BY BREAST CANCER

MacIndoe, J. H., Sherman, B. M., Granner, D. K., Ginsberg, B. H., University of Iowa, School of Medicine, Dept. of Medicine, *Jessup Hall, Iowa City, Iowa,* 52240, United States of America

There is substantial evidence that some hormone-dependent human breast cancers can synthesize androgens and/or estrogens from extracellular precursors. However, investigations into this and other potentially important aspects of sex steroid regulation have been hampered by difficulties with establishing such tumors in long-term tissue culture. We have observed that MCF-7, an established line of hormone-responsive human breast cancer cells, also has sex steroid biosynthetic capability. Furthermore, our studies suggest that both the formation and action of estrogens in this cell line may be subject to androgenic regulation.

The objectives of this proposal are to utilize MCF-7 cells to 1) further characterize the androgenic regulation of estrogen biosynthesis and action, 2) determine to what extent androgen formation and action may be modified by estrogens, and 3) test the hypothesis that the hormonal regulation of human breast cancer cells can be mediated by sex steroid derived from intracellular biosynthesis. These studies will involve the measurement of enzyme activities and steroid hormone receptors in cells incubated with androgens, estrogens, or their precursor steroids with and without various enzyme inhibitors, antiestrogens, and antiandrogens.

These studies are likely to provide a series of new insights into the hormonal regulation of human breast cancer cell growth which should eventually lead to the development of improved breast cancer therapies. Since it is likely that there are fundamental similarities between the hormonal regulation of malignant and healthy tissues, our data may also provide a better understanding of the basic mechanisms of steroid hormone action.

BIBLIOGRAPHIC REFERENCES: MacIndoe, John H., Estradiol Formation from Testosterone by Continuously Cultured Human Breast Cancer Cells, J. Clin. Endocrinol. Metab. 49:272, 1979.

89. STUDIES OF NORMAL AND MALIGNANT HUMAN BREAST EPITHELIUM IN VITRO

Blackstein, M. E., University of Toronto, Faculty of Medicine, Dept. of Anatomy (Histology), *92 College St., Toronto, Ontario,* M5S 1A8, Canada

We have developed a cell culture system which is capable of detecting colony formation derived from single cells both from normal human breast epithelium and malignant breast epithelium. The in vitro system requires a source of serum and has an absolute requirement for exogenously added insulin and hydrocortisone. In addition, a variety of other substances have been tested, including growth factors such as EpGF, LATS and a variety of polypeptide hormones and steroid hormones. To date, none of these other substances have had significant effect (other than thyroid hormone) on colony formation from either normal or malignant epithelium.

Studies on normal breast epithelium include interaction between the variety of cells present including foam cells and non-breast epithelial cells and their necessity in colony formation. A variety of other studies, including growth and differentiation as judged by induction of protein markers such as casein and lactalbumin and lactose synthetase production are under way. Studies on epithelial cells from normal women with a strong family or genetic background of breast cancer are being undertaken to attempt to determine if the genetic susceptibility to breast cancer resides within the breast genetic information or is exogenous (endocrine abnormalities). Studies on malignant breast epithelium include the ability to induce differentiation with a variety of substances and the nature of self renewal of the colony forming unit. Suspension culture of breast tumor cells can be most readily obtained when they occur from pleural effusion material. Using this as a model, we have been able to develop a collagen matrix system in which solid breast epithelial cells from the primary tumor are dissociated and aparently undergo selective growth with suppression of non-malignant and fibroblast contaminants. Studies are now under way to determine if such a system can be adapted to test in vitro drug and hormonal sensitivities.

90. CULTURE OF HUMAN NORMAL, DYSPLASTIC AND NEOPLASTIC BREAST TISSUE (SUBSTRATE EFFECTS)

Hallowes, R. C., Elias, J., Peachey, L. A., Jones, W., Riddle, P. N., Imperial Cancer Research Fund, Tissue Cell Relationships Laboratory, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

Previous studies (ICRF Scientific Report 1978 - report 166) showed that it was possible to dissociate normal human breast tissue into epithelial structures (organoids), to maintain and grow these on rat-tail collagen gels and to characterize the cell population present in the cultures.

During the past year, methods have been devised for suspending organoids or cell suspensions from organoids within the gel and for studying their cellular behavior. Organoids produced cellular sproutlets that extend into the surrounding gel, whereas single cells grew into horizontally arranged cellular plaques. Extension from intact lactiferous ducts to extralobular ducts was from the exposed ends rather than from along the length whereas from intralobular and terminal lobular alveolar ducts the extensions radiated from around the periphery. Changes were induced by the organoids, but not by cells, in the collagen gel which became more fibrillar. Similar extensions were not observed when equivalent organoids and cell preparations were suspended in soft agar or agarose and no interactions with these substrates were observed.

Time-lapse cinephotomicrography showed that the extensions resulted from the mobilization of an actively moving cell that purposefully entered the surrounding gel and was followed by cones or sheets of similar elongated cells that were adherent to each other. Cell divisions were observed in the sproutlets but were of too low frequency to account for the extension. Later a further cell population, consisting of cuboidal cells, appeared to colonize the cones already produced. Fibrillar condensation occurred within the gels and these appeared to form the pathways for the cellular extensions. Fibroblasts were not seen to have an active role in these processes.

Organoids have been dissociated from fibroadenomas and other dysplasias and were suspended in gels. Growth from fibroadenomas was mainly of elongated cells whereas those from lobular hyperplasia were from cuboidal cells. Electron microscopical and immunocytochemical studies indicate that the elongated cells are myoepithelial or basal cells, and the cuboidal are luminal epithelial cells.

Dissociates from breast carcinomas have also been suspended in collagen gel and three behavioral patterns were observed. (Text Abridged)

91. CHARACTERIZATION OF HUMAN MAMMARY CELLS

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We are developing an in vitro human mammary cell culture system to be used for studies of human cellular differenti-

ation and carcinogenesis. In particular, we will investigate: (1) the effects of potential growth promoters on human mammary epithelial cells; (2) the effects of hormones and cell substrates on expression of mammary-specific products (medium chain fatty acids, alpha-lactalbumin, casein); (3) properties that may differentiate between normal and malignant mammary epithelial cells (surface properties, tumor-specific antigens); and (4) metabolism of benzo-a-pyrene by these cells.

92. ASSAY FOR GENETIC DAMAGE TO MAMMARY GLAND CELLS

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OBJECTIVE: Devise or design an assay for detecting genetic damage or changes that may increase the rate of damage in mammary cells. General possibilities for assessing genetic damage might be the enumeration of cells with mutant phenotypes, determination of induced damage to DNA of individual cells or of populations of cells, or the enumeration of cells with evidence of mitotic disturbances. Such an assay would then be used to study the effects of age, diet, hormones, carcinogens, etc., on the incidence of genetic damage.

93. THY-1 DIFFERENTIATION ANTIGEN IN RAT MAMMARY TISSUE

Ritter, M., Pain, C., Rudland, P. S., Imperial Cancer Research Fund, Membrane Immunology Laboratory, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

We have demonstrated the presence of Thy-1 antigen on in vivo rat mammary tissue, using immunofluorescence on wax-embedded tissue sections. Thy-1 is found on ductal and alveolar myoepithelial cells of normal, pregnant and lactating animals (myoepithelial cells identified by Coomassie blue staining of myofilaments and by their position between the epithelial cells and the basement membrane). Thy-1 positive myoepithelial cells were also found in Dimethylbenzathracene and N-methylnitrosourea-induced mammary adenocarcinomas, and in adenocarcinomas derived from Rama 25 cells (cuboidal epithelial stem cell line).

Rat mammary cell lines were also studied by immunofluorescence. Rama 25, a stem cell line, was Thy-1 negative, whilst its myoepithelial derivative (Rama 29) was Thy-1 positive. Thy-1 antigen was also present on the elongated cells that appeared as fresh converts within several stem cell line cultures (Rama 75, 401 and 402). Clonal cell lines intermediate in morphology between the stem cell cuboidal line Rama 25 and the elongated myoepithelial-like cells of Rama 29 have been isolated. These intermediate types convert to myoepithelial-like cells when cultured at high frequency; during this conversion the appearance of surface Thy-1 correlates with the main morphological change and with the appearance of the extracellular matrix protein, fibronectin. Whether Thy-1 plays a causative role in the conversion process remains to be established.

94. THE USE OF PEANUT AGGLUTININ FOR THE STUDY OF CELL DIFFERENTIATION

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Fluorescently labelled peanut agglutinin (PNA) has been used to study cell differentiation. Specific binding of PNA to rat mammary gland increased as the gland matured, reaching a maximum in lactating animals. PNA was bound exclusively to the epithelial and not to the myoepithelial or mesenchymal cells.

In tissue culture, a rat mammary epithelial stem cell line (Rama 25) which can be stimulated to differentiate to alveolus-like or myoepithelial cells showed binding of PNA, but only to the secretory cells and not to unstimulated or myoepithelial cells. Fibroblast cultures did not bind PNA. Receptors on secretory cells were, however, mainly masked by sialic acid. Human breast sections, like those of the rat, showed labelling of the apical regions of the epithelial cells. Breast carcinomas that were morphologically differentiated showed more fluorescent labelling than undifferentiated tumors, which were weak or negative and confined to the cytoplasm.

In addition, PNA was used to investigate the maturation and differentiation of murine lymphoid cells. Cell suspensions were separated into PNA-plus and PNA-minus fractions by the fluorescence-activated cell sorter (FACS) and examined with various markers. CFU-s cells and thymocytes were PNA-plus, whereas mature T cells had most of their receptors masked by sialic acid. Early B cells possessed PNA receptors in an unmasked form but lost these completely as the cells matured from pre-B to peripheral B cells.

E. INSULINOMA AND PANCREATIC CELLS

95. EPITHELIAL-MESENCHYMAL INTERACTION IN ENDOCRINE TISSUE

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We will investigate the role of epithelial-mesenchymal interactions in the growth and functioning of certain hormone-secreting tissues, in particular, normal pancreatic islet cells and insulinoma cells. Our hypotheses are that adult epithelial cells and their associated mesenchymal cells are functionally interdependent and that the interrelationship is significant in the growth and/or functioning of both epithelium and mesenchymal cells. Furthermore, we believe that an understanding of these interactions will reveal new understandings of certain diseases such as malignancy, which may be aberrations of the epithelial-mesenchymal relationship.

We propose to develop a new in vitro technique for co-culturing multiple cell types. Essentially it involves culturing epithelial cells on rafts of reconstituted basement membrane and then floating the rafts over primary cultures of another cell type. We will assay the influence of the reconstituted basement membranes and the primary cultures of particular stromal cells on the plating efficiency, cloning efficiency, growth and tissue-specific functions of normal and malignant pancreatic islet cells. Our hypotheses are that there will be a dependence of the epithelial cells on the basement membrane and on primary cultures of stromal cells and that the malignant cells will be qualitatively and/or quantitatively less dependent on the mesenchymal factors.

96. ESTABLISHMENT OF INSULIN-SECRETING BETA CELL LINES

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Continuous beta cell cultures are needed for quantitative controlled experiments to a) elucidate the mechanisms of insulin biosynthesis and secretion; b) evaluate the immunological characteristics of beta cell constituents; and c) determine the mechanisms whereby specific cytotoxic chemicals and viruses damage beta cells selectively. Long-term maintenance, continuous passage, and the bulk propagation of pancreatic islet cells 'in vitro' have been hampered thus far by the limited replicative ability of the normal beta cell.

We are establishing reproducible animal models for induction of hyperplasia and transformation of the beta cell to establish permanent lines of insulin-producing lines of insulin-producing cells in tissue culture. Weanling rats and inbred mice receive injections of cortisone and gold-thioglucose to induce hyperplasia of the beta cells. Rodents than are administered ionizing irradiation and streptozotocin-nicotinamide. To detect hyperplastic and neoplastic changes in beta cells, blood from treated animals is monitored at intervals for glucose and assessed by radioimmunoasssay for elevated levels of insulin. Hyperplastic and transformed islet cells are isolated selectively using 1) differential attachment, 2) affinity columns, and 3) colloidal silica density gradients. Stimulation and establishment of beta cell lines are encouraged in vitro by use of complex media containing inhibitors for fibroblasts. The identification and assessment of the functional status of cultured beta cells is accomplished by aldehyde thionine and immunoperoxidase staining, and transmission electron microscopy.

97. TRANSCRIPTION OF INSULIN GENES IN ISOLATED NUCLEI FROM CULTURED INSULINOMA CELLS

Nose, K., Okamoto, H., Itoh, N., Toyama Medical & Pham. Univ., School of Medicine, Dept. of Biochemistry, *Sugitani, Toyama, Japan*

OBJECTIVE: In order to study transcriptional regulation of insulin genes in isolated nuclei, nuclei from cultured hamster insulinoma cells are transcribed either with endogenous or purified RNA polymerase.

APPROACH: Nuclei are isolated and transcribed in vitro in the presence of Hg-UTP to pick up de novo synthesized transcripts. The amount of insulin mRNA in the transcripts is quantitated by hybridization with 32p-insulin cDNA.

Conditions which affect the rate of transcription of insulin genes are examined.

98. ADAPTATION OF A HAMSTER INSULINOMA TO TISSUE CULTURE

Baum, S. G., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Dept. of Medicine, *1300 Morris Park Ave., Bronx, New York*, 10461, United States of America

This is part of a broader project. A summary of this subproject is not available.

99. IN VITRO ASSAY FOR INCREASED RISK FOR PANCREATIC CANCER

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In vitro research on the identification of increased risk for pancreatic cancer within families with pancreatic cancer with and without other solid tumors was based on the hypothesis that a cell considered normal by all criteria in a clinically normal individual, when grown in culture, would demonstrate such a mutant cancer-prone genotype. This hypothesis was based on in vitro research on the inborn errors of metabolism and the heritable colon cancer syndromes.

Dermal (fibroblast and epithelial) cultures were established from affected members, family members considered to be at increased risk and family members by marriage who did not have a family history of solid tumors as controls.

Increased in vitro tetraploidy has been observed in members at increased risk for pancreatic cancer in association with other solid tumors (offspring of affected persons). On the basis of this finding, increased in vitro tetraploidy may be useful in detecting risk for pancreatic cancer in families with heritable cancer syndromes involving more than 1 organ site. A cancer-prone gene expressed as solid tumors including pancreas in vivo may be detected in vitro by increased tetraploidy. Further research should demonstrate if increased in vitro tetraploidy identifies members at increased risk in families with pancreatic cancer.

100. PANCREATIC CARCINOMA IN CONTINUOUS CULTURE

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Human pancreatic carcinoma in continuous culture is being used as an experimental model to investigate 3 areas in all of which considerable work has been done in this laboratory: 1. Purification of a tumor-related antigen is underway. This antigen has been identified and appears to be immunologically identical to oncofetal antigen. We plan to develop a radioimmunoassay for this antigen and proceed to do large-scale testing of human sera. The effect of the antibody to the antigen on the growth and tumorigenicity of MIA PaCa-2 in nude mice will also be tested. 2. We have identified two proteolytic activites in cultured MIA PaCa-2, a plasminogen activator and a membrane protease. The latter will be purified and its role in tumorigenicity and tumor invasiveness will be assessed. 3. Extensive screening of chemotherapeutic agents will be performed on the cultured cells. Drugs found to have some effect on growth will be examined in depth for their effects on the cell cycle and various metabolic aspects of the cells.

Particular attention is being given to the potential role of glutamine antagonists as anti-pancreatic cancer agents.

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F. GASTROINTESTINAL CELLS

101. IN VITRO GENERATION OF IMMUNITY TO HUMAN COLON CANCER

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The purpose of this research program is to develop an in vitro immune response to human colon cancer cells using whole tumor cells, cell membranes, or soluble moieties from the cells. Early efforts in generating an in vitro immune response have been hampered by the secretion from the human colon cancer cell of soluble factors which suppress as well as stimulate lymphoid cells. The present approaches include a) developing a xenoimmunization model to characterize antigenic and immunogenic aspects of the tumor cells; b) characterizing the immunodeterminants detectable by a monoclonal antibody to human colon cancer; and c) using liposome carriers to elicit immune responses.

Xenogeneic intraperitoneal immunity generated in mice exhibited relative specificity towards colon cancer cells at day 7 post-immunization. The immunity localized in the peritoneal cavity. This xenoimmunity was used to characterize antigens (tumor cells, cell membranes, and liposome-antigen complexes) for in vitro studies. Cytotoxicity was generated by all antigen preparations except cell membranes. In the in vitro studies the same antigens were coincubated with normal mouse spleen cells; in vitro immune responses were generated as evidenced by the presence of specific blastogenesis of the primed spleen cells against a battery of human cancer cell targets. Finally, a monoclonal antibody to human colon cancer was used to immunoprecipitate a family of molecules from the tumor cells. Some of these molecules appear unique for colon cancer. These preliminary results suggest that the in vitro immunization method when adopted to human studies has the potential for selectively immunizing patient lymphocytes for subsequent reinfusion into the patient.

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102. DIFFERENTIATION-INDUCTION IN HUMAN COLON CANCER CELLS

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The three human colon carcinoma cell lines established at our center were studied for identification of new potential markers of differentiation. Three types of markers were investigated; these include glycogen, the Na ion- K ion- and Mg ion-stimulated adenosine triphosphatases (ATPase), and 11 purine metabolizing enzymes. The levels of these markers were determined in the clone A and clone D cells of the heterogeneous DLD-1 line, in HCT-15 cells, and in DLD-2 cells, and in their corresponding xenograft tumors in nude mice. Cultured clone A, clone D, HCT-15, and DLD-2 cells were treated with either N, N-dimethylformamide (DMF) or sodium butyrate in an attempt to modulate levels of glycogen and the purine metabolizing enzymes. Butyrate induced a significant increase in the level of glycogen in all cell types. Both DMF and butyrate modulated the activities of the various purine enzymes. The direction and extent of modulation was agent dependent, enzyme dependent, and cell-line dependent. Of potential interest is the greatly reduced adenosine deaminase activity determined in clone A or clone D cells following exposure to DMF. Experiments testing DMF in vivo against human colon tumor xenografts in nude mice were initiated. Toxicity levels were determined, and experiments are now in progress to determine whether the colon xenografts will respond to DMF treatments.

103. HETEROGENEITY OF HUMAN COLONIC CARCINOMA

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During the first two years of this project we have developed methodology for the growth of human colonic cacinoma on feeder layers of confluent fibroblasts. This methodoloy has resulted in the long-term culture of 31/37 attempted sppcimens. We have identified 3 morphological patterns of growth in primary specimens which appear to be associated wth subpopulations of cells which have been isolated from 4 different carcinomas. These subpopulations have a wide range of malignant potential as assessed by tumorigenicity in nude mice and other in vitro criteria normally associated with malignancy. In this renewal application we propose to improve our culture methods by determining the optimal cell type for feeder layers, identifying growth factor(s) produced by feeder layers and increasing inocula of cultures by employing purified rather than unseparated malignant cells containing other cell types. These improvemnts may eventually lead to the development of an in vitro screening system of possible therapeutic modes for individual patients and will improve the chances of isolating subpopulations of cells from individual tumors. Isolated subpopulations of cells will be extensively characterized in order to determine whether they are developed by reversible differentiation processes or by irreversible mutation. Determination of the route by which subpopulations of cells with different malignant potential are produced in individual tumors will have important effects on the future development of therapeutic strategies for colon cancer.

104. ANALYSIS OF TUMOR PROGRESSION IN LIVER CELLS IN VITRO

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Hepatocarcinogenesis in vivo is now recognized to involve a complex, usually slowly developing sequence of linked cellular reactions that are entrained by a rapid initiating event and culminated by development of malignant lesions. Initiation is necessary, but not sufficient, to cause neoplastic transformation of hepatic epithelial cells. Separating the appearance of malignant tumors from the initiating event are a series of successive phenotypic alterations in the population of cells at risk that comprise the phases of tumor progression. Tumor progression may be viewed as a biological phenomenon essential to neoplastic transformation, during which initiated cells progressively develop phenotypic modifications that allow their selective multiplication. We have developed an in vitro transformation system, employing cultured hepatic epithelial cells, that reproduces a sequential process of tumor progression comparable to that in vivo. It is the goal of this proposed project to elucidate the mechanisms underlying the major cellular phenotypic variations arising during tumor progression by studying this process in transforming liver cells in vitro. Phenotypic variants studied will include a) increased resistance to the cytotoxic action of the carcinogen, b) more rapid and less regulated growth, and c) karyotypic instability. In addition the role of stem line selection and augmention in tumor progression will be analyzed.

G. GENITOURINARY CELLS

105. STUDIES OF SEX STEROID RECEPTORS IN PROSTATIC CARCINOMA TISSUE

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OBJECTIVE: The assessment of the presence and activity of sex steroid receptors in prostatic carcinoma tissue in order to develop a model system to predict the response of patients to hormonal manipulation.

APPROACH: Two assays are under development. a) Cell culture assay: A single cell suspension is obtained by enzymatic dissociation of prostatic carcinoma tissue during prostatic resection or biopsy. The acid phosphatase produced by these cells, in the absence and presence of androgens and estrogens added to the culture medium, is compared. The in vitro behavior of the patients' prostatic carcinoma cells is compared with their in vivo reaction to endocrine manipulation. b) Characterization of cytosol and nuclear androgen receptors: Protamine sulphate precipitates of standard nuclear and cytosol fractions of prostatic tissue are obtained. Binding kinetics and substrate specificities of these receptor fractions are determined, using 3H-dihydrotestosterone (3H-DHT) and 3H-methyltrienolone (3H-R1881) as ligands. The isoelectric focusing patterns of the receptor proteins are determined.

PROGRESS: a) Cell cultures: In cultures from 32 of 65 patients, acid phosphatase levels were higher in the presence of androgens than in their absence. In 24, there was no effect of androgens on this enzyme level, while in 9, androgens were associated with lower enzyme levels than in the control cultures. Similarly, there were 3 patterns of response of these cells to estrogens. In 30 of 36 cultures, estrogens were associated with either no effect or a fall in acid phosphatase levels. However, in 6 incubations, estrogens were associated with higher enzyme levels than in the control incubations. There has been good correlation between these cultures and the in vivo response to endocrine manipulation in 9 patients. c) Androgen receptors: Preliminary studies have shown that the protamine sulphate precipitates of both subcellular fractions bind both 3H-DHT and 3H-R1881 with high affinity. The specificity of these receptors differs from that of serum sex hormone binding globulin (SHBG) in that the affinity for DHT is 10 to the third power the affinity for estradiol. Results of isoelectric focusing reveal that the receptors have different isoelectric points from SHBG.

106. CHARACTERIZATION OF CELLS GROWN FROM HUMAN PROSTATE

Merchant, D. J., Johnson, A. H., Devine, P. C., Clarke, S. M., Ladaga, L. E., Eastern Virginia Medical School, School of Medicine, Dept. of Microbiology & Immunology, *358 Mowbray Arch, Norfolk, Virginia*, 23507, United States of America

The proposed program is intended to define biochemical/genetic markers for human prostatic acinar epithelial cells to aid in the identification of cells grown in tissue culture. It further is intended to test the primary culture system developed for prostatic TUR specimens for its practical usefulness in defining therapeutic sensitivities in the BPH or carcinoma of individual patients or for use as a prognostic tool.

107. ANDROGEN-RESPONSIVE PROSTATE EPITHELIAL CELLS

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An androgen-dependent, prostate-specific protein (prostatic binding protein; PBP) was isolated from rat ventral prostate cytosol and antiserum against it was prepared in rabbits. Studies revealed that PBP is present specifically in the epithelial cell fraction of primary cell cultures from prostate. Fibroblast-like cells contain no PBP. Therefore, PBP is an excellent marker for identification of prostate epithelial cells. The synthesis and secretion of PBP by prostate epithelial cells in primary cultures was monitored by labeling with (35S)methionine, reaction with anti-PBP serum and precipitation of the immune complex with Staphylococcus aureus containing Protein A. Cultured epithelial cells synthesize and secrete significant levels of (35S)PBP for up to 7 days in culture. The rate of synthesis is highest on day 1 and decreases daily during the 7 day period.

Synthesis and secretion of polyamines were studied as a potential marker of function of prostate epithelial cells. Polyamines were labeled with (3H)ornithine from the culture medium and then isolated by ion exchange chromatography. The (3H)polyamines were derivatized and the spermidine (SD), spermine (SP) and putrescine (PT) separated by thin layer chromatography. Individual (3H)polyamines were quantitated in cells and medium. Appearance of total (3H)polyamines in the medium peaks on day 6 of culture. The bulk of polyamines secreted into the medium and in cell extracts is putrescine. However, the SD plus SP:PT ratio peaks on day 2 of culture and declines rapidly. Fibroblast-like cells secrete a very low ratio of SD plus SP:PT. We propose that a high ratio of SD plus SP:PT in the culture medium is indicative of prostate epithelial cell function.

108. TISSUE CULTURE OF GONADOTROPIN RESPONSIVE LEYDIG TUMOR CELLS

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OBJECTIVE: The major aims of this project are to establish cultures of gonadotropin responsive Leydig tumor cells (derived from the M5480 Leydig cell tumor) and to study the factors that affect their growth and expression of differentiated function.

APPROACH AND PROGRESS: Cell cultures have been established by the culture-animal passage technique. The cultured cells retain their ability to bind human choriogonadotropin (hCG) and to respond to it via increased steroid production. Preliminary results indicate that epidermal growth factor stimulates cell multiplication and reduces the number of hCG receptors in the cultured cells.

109. MOUSE TERATOMA AND DERIVED CELLS STUDIES

Chung, A. E., Braginski, J. E., Madhav, R., Lewis, M., University of Pittsburgh, School of Arts & Sciences, Dept. of Biological Sciences, *4200 5th Ave., Pittsburgh, Pennsylvania*, 15260, United States of America

The long-term objective of this project is to study the mechanism of differentiation of mouse embryonal carcinoma cells. The clonal cell lines PCC4-F, PCC4-aza-1, and PCao will be used for these studies. Emphasis will be placed on the in vitro differentiation of these cell lines. The specific approaches will be: (1) Examination of the influence of embryonal carcinoma cell cytoplasm on the gene expression in differentiated cells and the influence of the cytoplasm of differentiated cells on the multipotentiality of embryonal carcinoma cells, (2) comparison of the properties of the embryonal carcinoma cell with embryonal carcinoma derived lines PFHR-9 and M1536-B3, (3) development of an in vitro myogenic differentiation pathway and (4) exploration of the effect of a limited number of extrinsic factors on the differentiation of the embryonal carcinoma cell.

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110. DRUG RESISTANCE IN MAMMALIAN CELLS

Ling, V., Ontario Cancer Inst., Biological Research Division, *500 Sherbourne St., Toronto, Ontario*, M4X 1K9, Canada

OBJECTIVE: To study mechanisms of drug resistance in Chinese hamster ovary (CHO) tissue culture cells. To examine human tumor cells for similar resistance.

APPROACH: 1) Mutants resistant to colchicine have been isolated from CHO cells. These cells display pleiotropic cross-resistance to a variety of compounds including chemotherapeutic drugs such as adriamycin, daunomycin and vinblastine. By drug uptake studies, the mechanism of resistance appears to result from reduced drug permeability. The chemical composition of plasma membranes of mutant and parental cells has been analyzed. 2) Mutants resistant to daunomycin have been isolated from CHO cells. They also display cross-resistance to other drugs similar to (1) above; however, the pattern of resistance is different from the colchicine-resistant lines. These are probably membrane mutants different from (1). 3) Mutants resistant to melphalan have been isolated. The mechanism of resistance is currently being investigated.

111. ESTABLISHMENT AND COMPARISON OF THE VALUE OF TWO ASSAY METHODS FOR CARCINOMA OF THE OVARY

Boesen, E. A., Corringham, R. E., Royal Free Hospital, Dept. of Oncology, *Pond St., Hampstead, London NW3 2QG, England, United Kingdom*

OBJECTIVE: The purpose of this study is to develop an in vitro method of assaying the sensitivity of carcinoma of the ovary to cytotoxic drugs and to compare this method with a tumor xenograft system in immune deprived mice.

APPROACH: Tumor cells will be obtained from ascites and effusions and from surgical specimens from patients with carcinoma of the ovary. In vitro method: After preparation the tumor cells will be cultured in soft agar. When cloning of the tumor cells has been successfully established further tumor cells from the same specimen of ascitic fluid will be incubated with various cytotoxic drugs prior to plating and the cloning ability of these tumor cells assessed. The results would be correlated with the response to cytotoxic drugs observed in vivo. Assessment will be repeated during the clinical course of the disease. Xenograft method: Tumor cells from the same sample will be implanted in immune deprived mice and an assessment made of the sensitivity to cytotoxic drugs. A comparison between the two methods can then be made.

112. CULTURE AND TRANSFORMATION OF RAT OVARIAN CELLS

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OBJECTIVE: This study was undertaken to examine the susceptibility of rat ovarian cells to C-type RNA tumor viruses, and, if successful, to establish a model for virally induced ovarian cancer.

APPROACH: Cells from young adult rats were cultured and specific cell types were identified by morphology and histochemical characterization of steroid dehydrogenases. Work is under way to establish methods to grow and characterize pure cultures of specific ovarian cell types. Subsequently, cultures of pure cell types will be examined for susceptibility to murine sarcoma viruses, and any transformed lines obtained will be examined in vivo for resemblance to specific groups of ovarian carcinomas in humans. The lines will be characterized with respect to ultrastructure and steroidogenic activity, as determined histochemically and biochemically.

PROGRESS: Ovarian surface epithelium has been cultured in pure form and characterized ultrastructurally and histochemically (positive for 17-beta-HSDH and negative for delta-5,3-beta-HSDH). Transformed tumorigenic lines have been obtained following Kirsten sarcoma virus infection. These lines resemble normal surface epithelium by marked 17-beta-HSDH activity and also show slight delta-5,3-beta-HSDH activity.

113. HORMONAL REGULATION OF CULTURED ENDOMETRIAL CELLS

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A technique to culture epithelial rabbit endometrial cells in chemically defined medium has been developed. Estrogens were found to promote cell division, while progesterone had an opposite effect. These cultured cells were found to be either noncycling (G0) or cycling. Both cell subpopulations were isolated and it was found that the G0 cells were the target of estrogens. Interaction between progesterone and the cycling cells was necessary to obtain an antagonistic effect on the estrogen action on the G0 cells, apparently due to the production of a diffusible factor. A similar factor, probably a protein, appears to be produced by high density cultures, which do not respond to estrogen stimulation.

Estradiol and progesterone decrease the number of estrogen receptors in cultures, apparently by different mechanisms of action. We will continue to study our experimental system by analyzing the mechanism of action of ovarian sex hormones, epidermal growth factor and prostaglandin F-2-alpha in regulating proliferation, cell cycle, blastokinin synthesis, and hormone receptors. The endpoint of our research is to improve our comprehension of the mechanism by which sex hormones regulate proliferation and differentiation of endometrium. It is expected that the study of our hormone-responsive system will help to advance the understanding of the etiology and pathogenesis of endometrial carcinoma in particular and hormone-related cancer in general.

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114. VAGINAL AND CERVICAL EPITHELIAL CELL CULTURE MODEL

Rice, R. H., Harvard University, Boston Campus, School of Public Health, Dept. of Physiology, *55 Shattuck St., Boston, Massachusetts*, 02115, United States of America

This project involves cultivation of cells derived from normal and abnormal female lower genital tract epithelia. The cells are serially passaged with support from feeder layers of lethally irradiated mouse 3T3 cells (the Rheinwald-Green system). The overall objectives for this culture system are: 1) to study the growth and differentiation of the epithelial cells and 2) to investigate the etiology and progression of cervical carcinoma. So far, the results obtained by our optimization of the growth of normal cells by varying concentration of factors such as hydrocortisone cholera toxin, epidermal growth factor as well as feeder layer density and cell type are consistent with a recent report that the cells grow well (Stanley and Parkinson (1979) Int. J. Cancer 24:407). Human cervical cells at varying stages of dysplasia or carcinoma will be propagated and examined for dependence upon growth factors and feeder support and for expression of differentiated character (keratins, cross-linked envelopes, cytochrome P450, estrogen receptors). In addition to permitting characterization of the cellular differentiation and possibly mechanisms of tumor progression, the results of this work may also indicate to what extent keratinocytes from different anatomical locations (epidermis, cervix) differ in culture.

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115. CLONOGENIC CELLS FROM HUMAN TUMORS

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OBJECTIVE: The project objective is to investigate the biological characteristics of in vitro clonogenic cells from human tumors. Those tumors chosen for study are transitional cell carcinoma of the bladder (TCC) and malignant effusions from metastatic carcinoma.

APPROACH: The approach is to analyze factors (in situ-derived and in vitro-derived) which determine clonogenicity and in addition to assess characteristics such as kinetics, in vitro drug sensitivity and renewal capacity of those cells which are clonogenic.

PROGRESS: Clonogenic assays have been developed for cells derived from human TCC and from carcinoma malignant effusions. The in vitro characteristics of these cells correlate well with the status of the tumor in situ. Evidence has been accumulated to show that host-cell interaction and the choice of semi-solid support are important determinants of clonogenicity. Host-cell interaction is mediated through a step requiring prostaglandin synthesis by intra-tumor macrophages. An assay has been developed to measure the self-renewal probability of clonogenic ovarian carcinoma clonogenic cells. Characterization of this procedure is complete and data are now being collected to determine the prognostic indication of this measure of biological aggressiveness.

116. TRANSFORMATION IN VITRO OF HUMAN URINARY TRACT CELLS

Reznikoff, C. A., DeMars, R., Croft, W., Norback, D., Santhanan, S., University of Wisconsin, Madison Campus, Medical School, Dept. of Genetics, *450 N. Randall Ave., Madison, Wisconsin*, 53706, United States of America

The primary objectives of this research project are to establish and characterize a bona fide strain of human urothelial cells and to induce mutations to thioguanine resistance and to morphological transformation in vitro in these cells using chemical carcinogens and other mutagenic treatments, such as UV light.

Cultures of normal human transitional epithelial cells will be initiated using explants of embryonic bladder and of postnatal ureter and bladder obtained from surgery. Several different culture media will be tested to determine which media best support the growth and the maintenance of normal human urothelial cells in culture.

The morphological and growth characteristics of cells grown from primary explants will be studied at each stage of adaptation to tissue culture to determine (1) whether the cells cultured are truly urothelial cells and (2) whether the cell strains derived have useful in vitro growth and morphological properties. Experiments to characterize cells and to determine their usefulness will include scanning and transmission electron microscopy, as well as the measurement of various in vitro growth parameters.

Several epithelioid cell strains have already been derived from urothelial explants and one of these has been successfully used for quantitative mutation experiments. We are in the process of completing the characterization of these cell strains and we plan to use them in several preliminary transformation assays.

117. PROLIFERATIVE POTENTIAL OF CULTURED EPITHELIAL CELLS

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Using epithelial cells from primary human embryonic kidney cultures (HEK) and Madin-Darby canine kidney epithelial cells, we plan to study cell interactions with substrates composed of basement membrane components or of lectins. One of the basement membrane components we are investigating is laminin, which has been isolated from a transplantable mouse tumor in the laboratory of Dr. George Martin at N.I.H. We plan to elucidate the carbohydrate composition of laminin and investigate its interaction with various lectins. We will likewise characterize the surface of the epithelial cells with respect to which lectins they bind. We will then study the mechanism of binding of epithelial cells to laminin-coated substrates by trying to block binding with lectins which interact with laminin but not to the epithelial cells and vice versa. If we can find lectins which block cell binding to substrate, this will provide information about what kinds of carbohydrate moieties, either on the cell surface or on the laminin, are involved in the cell adhesion. We will then be able to see whether the adhesion can be blocked by appropriate oligosaccharide. We are also studying the adhesion of epithelial cells to substrates covered with lectins. These interactions will serve as models for studies on the adhesion of epithelial cells to basement membrane components.

118. ETIOLOGY OF UROGENITAL TUMORS

Rapp, F., Drago, J., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Microbiology, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

The overall objective of this project is to attempt to determine the etiology of urogenital tumors. Specifically, this will be approached by studying biological and immunological properties of cloned lines of human cytomegalovirus-transformed human cells.

One of the areas of current interest is the mechanism by which cytomegalovirus-transformed cells lose their viral antigens and become more oncogenic during prolonged in vitro cultivation.

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119. REGULATION OF 10 NM FILAMENT ASSEMBLY IN NON-MUSCLE CELLS

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The overall objective of this research is to extend our ongoing studies of the structure, function and biochemistry of 10 nm filaments in cultured cells. A technique has been developed in our laboratory which allows the rapid isolation of juxtanuclear birefringent caps of 10 nm filaments (FC) which form in baby hamster kidney (BHK-21) cells during the early stages of cell spreading and following the addition of colchicine

to the culture medium. The isolated FC retain their birefringence and are greatly enriched with 10 nm filaments. Analysis by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis has demonstrated that up to 90 percent of the total BHK-21 FC protein consists of two major subunits of molecular weight 54,000 and 55,000. The yield of protein related to 10 nm filaments is approximately 2.5 percent of total cell protein. Initial attempts have been made to isolate FC from other types of mammalian cells and preliminary analyses indicate similarities as well as differences from one cell system to the next. These differences may reflect the different functions of 10 nm filaments in different cell types.

H. MELANOMA AND SKIN CELLS

120. INVESTIGATIONS OF A MITOGENIC FACTOR FROM HUMAN CANCER CELLS

Davidson, L. A., Baker, R. S., MeherHomji, K., University of Sydney, School of Public Health & Tropical Medicine, *Sydney, N.S.W. 2006, Australia*

OBJECTIVE: To determine the biochemical properties of a growth stimulating factor or factors found in fresh human cancer biopsies and produced by cultured cancer cells in vitro, and to establish the relation between this/these factor(s) and other known mitotic stimulators found in cells.

APPROACH: A bioassay for growth stimulating factors has been developed in this laboratory. The method employs a line of human melanoma cells growing in agar suspension and found previously to be sensitive to factors released from cancer cells and tissue biopsies.

Cancer cells and tissues obtained by biopsy were homogenised and stored frozen at minus 90 degrees C before assay.

Biochemical investigations involving Sephadex column chromatography will be carried out on selected specimens showing greatest biological activity. Partially purified fractions will be further subjected to isoelectric focussing and to polyacrylamide gel electrophoresis. Purified material will be tested for its carbohydrate and protein content, and at each purification step, activity will be monitored by bioassay.

121. CELL CYCLE DEPENDENCY OF CELL SURFACE RECEPTORS

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The mechanism and physiological function of cell cycle dependent changes in cell surface receptors will be investigated in two different systems: 1) MSH-receptors of melanoma cells, and 2) hapten-binding cell surface immunoglobulins (sIg) of myeloma cells. 1. We have previously shown that the expression of MSH receptors in melanoma cells is dependent on the cell cycle; it is maximal in the G2 phase. Our major objective now is to determine the mechanism and physiological role of cell cycle dependency of MSH receptors and to investigate how the responsiveness of melanoma cells to MSH is regulated by growth controls. Cell cycle and MSH-receptor content of melanoma cells, cultured in serum-containing and chemically defined medium, will be analyzed by microfluorimetry and flow cytometry of cells labeled with MSH. The effect of growth controls on the expression of MSH receptors and hormone-induced melanogenesis will be studied. Also, we want to develop a method to prevent cell cycle-dependent evasion of melanoma cells from site-directed (MSH-receptor mediated) chemotherapy of melanomas. 2. We have found that the number of hapten-binding sIg (315) changes during the cell cycle of MOPC-315 plasmacytoma cells. We now want to characterize the nature of sIg (315) and elucidate the mechanism of cell cycle related changes in sIg (315). H and I chains of sIg (315) will be chemically characterized. Mono- and multi-valent DNP-carrier conjugates will be used for labeling DNP binding cells; cell cycle of labeled cells will be analyzed by microfluorimetry and flow cytometry. The effects of growth controls on the expression of antigen-binding receptors will be studied.

122. GENE ACTION AND CELLULAR DIFFERENTIATION IN CULTURE

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This work is aimed at exploring regulatory mechanisms involved in normal differentiation and malignant transformation, using the B16 melanoma model system involving melanotic and amelanotic malignant clones and amelanotic nonmalignant clones derived from the melanotic clone after growth and maintenance in the presence of 1 microgram/ml of 5-bromodeoxyuridine (BrdUrd). The BrdUrd clone can immunize syngeneic mice against the parental tumor. By using hyperimmune mouse antisera to the BrdUrd-grown clone and to irradiated parental malignant cells we are comparing glycoproteins and proteins of the 2 cell types by immunoprecipitation, immuodiffusion, immunofluorescence and cytotoxicity. Peritoneal exudate cells from immune animals can neutralize the tumorigenic ability of the malignant cells when coinjected into syngeneic mice. Macrophage:T cell cooperation appears to be involved. Delineation of the T cell type is under way. Peritoneal exudate cells from mice immunized with heat-killed BCG also neutralize malignant cells. The effector cells appear to be macrophages. We have shown that correlation between plasminogen activator (PA) activity and tumorigenicity and between inhibition of PA activity and tumorigenicity need not exist. We are examining the mechanism by which 5-azacytidine and other regulators of differentiated function operate. These approaches should lead to further understanding of regulatory mechanisms in malignancy, immuogenicity and melanogenesis.

123. CULTURED EPIDERMAL CELLS AS A MODEL FOR SKIN CARCINOGENESIS

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The majority of human neoplasms are thought to be due to environmental factors. There is overwhelming evidence that skin cancer, one of the most commonly occurring human cancers, is usually due to exogenous insult. The cells that are most likely to be progenitor cells for this carcinoma are the basal cells, which are rapidly dividing and/or differentiating, and as such make excellent target cells for carcinogenic agents. The purpose of this work is (1) to develop an in vitro system consisting of dividing, basal cells isolated from hamster epidermis, (2) to induce these cells to undergo neoplastic transformation in vitro following treatment with environmental carcinogens and/or promoters, (3) to determine whether the two-stage model of carcinogenesis can be extended to Syrian hamsters who are not sensitive to phorbol ester promotion of epidermal carcinogenesis.

BIBLIOGRAPHIC REFERENCES: Barrett, J.C., and Sisskin, E.E.: Studies on Why 12-0-Tetradecanoylphorbol-13-acetate Does Not Promote Epidermal Carcinogenesis in Hamsters. In E. Pullman, P.O.P. Ts'o and H. Gelboin (Eds).: Carcinogenesis: Fundamental Mechanisms and Environmental Effects. D. Reidel Publishing Co., Dordrecht, Holland (1980), in press.

I. OTHER SPECIFIC CELL TYPES

124. EPSTEIN-BARR VIRUS AND NASOPHARYNGEAL CARCINOMA

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OBJECTIVES: Studies on the infection by Epstein-Barr virus (EBV) of normal human epithelial cells, particularly nasopharyngeal cells; cellular hybridization studies with an HR-1 cell line containing one EBV genome per cell; studies on the transformation of normal human primary epithelial cells with EBV by a nuclear exchange procedure; studies on in vivo cell fusion of EBV genome positive lymphoid cells and nasopharyngeal cells.

125. CORRELATION BETWEEN IN VITRO AND IN VIVO ACTIVITY IN THE SAME EXPERIMENTAL TUMOR

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OBJECTIVE: The aim of our work was to study drug effects on the in vitro and in vivo cell variants of Lewis lung carcinoma (3LL), differing in growth rate, oncogenic ability, and metastatic potential.

APPROACH: An in vitro-in vivo model system derived from 3LL tumor was obtained (Zupi et al., Brit. J. Cancer, 1980 41, supp. IV:309) and the different cell variants were studied for their response to ADM, BLM, HOU and some antimetastatic drugs (ICRF-159).

PROGRESS: a) Characterization of cell variants - Three in vivo and two in vitro lines were characterized for their oncogenicity (TD50), metastatic potential and growth rate. Results indicate that the in vivo and in vitro lines differ in all those parameters; in particular our interest has been focused on the metastatic potential. In fact, the in vitro line BC215 and the in vivo subline BM21548 show a ten times lower metastatic ability than the C108 line and BM21548 subline, respectively. Whereas flow cytometric studies indicate a tetraploid DNA content and a very similar DNA modal value in both primary and metastatic tumors, they also show a hyperdiploid DNA content for the BM21548 subline. b) Drug effects on different cell variants - The in vitro lines (C108, BC215) were studied for their sensitivity to ADM, BLM, HOU, ICRF-159. Results obtained indicate that the line with the highest metastatic potential (C108) is the one most sensitive to the ICRF-159 treatment (M. Caputo et al., Procs. Met. Confer. EORTC Metas. Proj. Group - The Hague: M. Nijhoff Publ., 1980). The in vivo sublines were studied for their response to ADM and BLM. The results obtained indicate that these lines elicit a different response also when different drug schedules are employed (C. Greco et al., Eur. J. Cancer, 1980 16: 1251).

126. CLONAL ANALYSIS OF BIOLOGICAL HETEROGENEITY IN PRENEOPLASTIC SALIVARY GLAND CELL LINES

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We have previously described (ICRF Scientific Report 1978 - report 137) several preneoplastic epithelial cell lines derived from foci of abnormal Stage III cells tranformed by chemical carcinogens in vitro. Stage III foci in primary culture are heterogeneous in their capacity for growth in serially passaged culture and in their potential for transition to Stage IV, characterized by the ability to produce tumors in syngeneic mice. We are attempting to obtain evidence for the preliminary indication that heterogeneity is influenced by conditions early in the culture history, such as carcinogen treatment protocol.

We are also studying the heterogeneity within and between different lines of preneoplastic epitheial cells during their progression to Stage IV. We have isolated a number of sister clones from early passages of each cell line, in order to compare characteristics and assess the role of clonal selection in the acquisition of tumorigenicity. The two best studied lines, CSG 205 clone 2B1 and CSG 211, differ in two respects, their capacity for growth in soft agar and their response to the tumor promoter, TPA. CSG 205/2B1 produces carcinomas at about passage (P) 20, but at P33 still shows no growth in soft agar into which 10 to the power 6 cells have been seeded per 5cm dish with feeder cells. In contrast, CSG 211 acquires the capacity to grow in soft agar (colony forming efficiency equals 0.07 percent) under the same conditions, at about the passage level that first produces carcinomas (P27).

Weekly treatment with TPA stimulates growth of preneoplastic CSG 205/2B1 compared to parallel solvent-treated cultures but does not affect the timing of Stage IV change. In contrast, TPA inhibits growth in CSG 211 but preliminary evidence suggests that it may accelerate the onset of Stage IV in this cell line relative to parallel sovent-treated cultures. Biological behavior can be related to chromosome complement in the parental lines (see Report 132) and to sister clones derived from them. The aim is to identify specific, possibly necessary changes involved in the evolution of malignancy and associated in vitro markers of transformation.

127. IN VITRO AND IN VIVO STUDIES OF RETINOBLASTOMA

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The establishment of a number of cultured cell lines of retinoblastoma during the past several years has opened the way for a new program of research with this malignancy. It has made possible a short-term, but effective and dependable, ocular model of the disease in immunosuppressed rabbits. Various retinoblastoma cell lines will be studied in vitro and in our animal model for the range of cellular sensitivity to the photodynamic activity of natural and synthetic porphyrins such as hematoporphyrin and tetraphenylporphine sulfonate. Retinoblastoma karyology of the tumor, and of family member lymphocytes, will be examined for possible distinguishing chromosome abnormalities. Cell lines will be used in animal studies to determine their ability to develop metastatic spread. They will also be examined for antigenic and biochemical characteristics that may help establish the cell of origin in the retina.

BIBLIOGRAPHIC REFERENCES: Sery, T.W.: Photodynamic Killing of Retinoblastoma Cells with Hematoporphyrin and Light. Cancer Research 39:96-100, 1979.

128. PROPERTIES OF METASTASIZED CELLS

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OBJECTIVE: To select cell lines with a high capacity for metastasis and study their properties, with special emphasis on the membrane. To evaluate the sensitivity of these cell lines to various drugs.

METHOD: We are currently working with non-transformed BHK cells, or BHK cells transformed by HaSV, and the L929 cell line. Various spontaneous transformers and cell lines capable of growth in agar in the presence of 2-deoxyglucose are used. Clones obtained are being studied by such diverse methods as: tumorigenicity study, enzymatic radioiodination, hexose penetration, etc. The effect of proteolytic enzyme inhibitors of hexose transport will also be studied.

III. SPECIFIC APPLICATIONS OF TISSUE CULTURE TECHNIQUES

A. EVALUATION OF DRUGS AND RADIATION DOSES IN CELL CULTURES

129. MONITORING OF DRUG EFFECTS IN SHORT-TERM CULTURES OF HUMAN TUMORS

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OBJECTIVES: 1. To assess limits and perspectives of an in vitro chemosensitivity test, based on the interference of drugs in nucleic acid precursor incorporation, in predicting clinical response to chemotherapy of individual tumors. 2. To compare the in vitro chemosensitivity data obtained with the antimetabolic assay and with the cloning assay on the same tumor.

APPROACH: Tumor material obtained by surgical biopsy is divided into two portions: for routine histologic examination and the other one for in vitro studies. Immediately after surgery, besides chemosensitivity assay, for each tumor also the kinetic determination is carried out (3H thymidine labeling index). The antimetabolic assay is carried out on small fragments or cell suspensions incubated for 3 hours in the presence of different concentrations of the drugs used in the clinical trials. During the last hour of incubation, 3H thymidine and 3H uridine are added to the culture medium. At the end of incubation nucleic acids are extracted according to the method of Schnider modified for our purposes; the radioactive precursor incorporation is expressed as total dpm for cell suspensions and as fractional incorporation for fragments. The in vitro effect of the drugs on nucleic acid precursor incorporation is expressed as percent variation from control values. In vitro sensitivity indexes are defined by the analysis of in vitro sensitivity and clinical response of the same tumor taking into account also the kinetic characteristics. The clonogenic assay is carried out ac-

cording to the method of Hamburger and Salmon. The relationship between the in vitro and in vivo results is statistically analyzed by the chi-square test and the actuarial life method.

PROGRESS: By using the antimetabolic assay, the analysis of the relationship between in vivo and in vitro chemosensitivity carried out on 78 cases, including non-Hodgkin's lymphomas (NHL), breast and testicular cancers, showed a high predictive accuracy of sensitivity (90%) and resistance (61%) with a significant correlation between in vitro and in vivo data in 76% of the cases (p less than 0.0005). More particularly, in NHL (44 tumors) the in vitro sensitivity was related to clinical complete remission (p less than 0.005), disease-free survival (DFS) (p less than 0.05), and survival (p less than 0.0005). (Text Abridged.)

130. DIFFERENTIAL DRUG SENSITIVITIES OF HUMAN TUMOR CELLS

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Because the currently used anticancer drugs are generally not selective enough to cause total tumor eradication, we often find that the initially successful chemotherapy is followed by recurrence of tumor growth, possibly due to the development of drug resistance. Such expressed or acquired resistance may be the result of differences in cell kinetics or in the cell's ability to repair drug induced damage, or of the presence of a number of different clones of cells within the tumor, all having different drug sensitivities. It will be our goal to establish several different human tumor cell lines from a single biopsy specimen (and in a variety of tumors); to characterize their in vitro growth kinetics; to determine differential sensitivities to a specific drug, and cross-sensitivities and resistance to other drugs; and to determine the causes of these responses when possible. The preliminary results reported in this proposal and the plans for future experiments illustrate that such studies may suggest useful drug combinations and the time sequence of administration for overcoming these forms of resistance.

131. EFFECT OF CALIFORNIUM-252 NEUTRONS ON HYPOXIC HELA CELLS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

132. DEVELOPMENT OF A CYTOLOGIC TECHNIQUE FOR THE ESTIMATION OF CELLULAR RADIATION SURVIVAL PARAMETERS

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OBJECTIVE: Attempts are being made to correlate the frequency of micronuclei with variations in the survival curve parameters of cell populations of different radiosensitivities.

APPROACH: Sensitivity variation in cell cultures is induced by oxygen or hypoxic radiosensitizers in varying concentrations. In parallel experiments, the frequency of micronuclei is determined and the survival curve parameters are established after irradiation with varying X-ray doses. The correlation between the cytologic and survival curve parameters is analyzed with multiple linear regression. The regression constants established by these analyses are tested in model experiments with cell cultures of predetermined, known sensitivity variations. In complementary experiments similar tests will be made using biopsy material from experimental tumors growing in vivo.

133. X-RAY DOSE-RATE EFFECTS ON CELL KINETICS

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The objective of this study is to obtain a detailed picture of cellular kinetics and lethality under conditions of continuous irradiation and to investigate whether and how the effects of continuous irradiation can be predictably reproduced by fractionated treatment in four different in vitro cell lines.

The dose-rate effect due primarily to the accumulation of radiation damage and repair was studied by continuous irradiation at various dose rates at temperatures which slow down or prevent the movement of cells through the cycle. These studies will also include stationary cell populations in plateau phase.

Dose-rate effects due to differences in the characteristics of the mitotic cycle will be studied by incubating cells during irradiation at temperatures between 28 degrees C and 37 degrees C, a procedure which essentially does not affect the ability of cells to accumulate and repair radiation damage, but which multiplies the duration of the mitotic cycle several times.

The potential advantages of combining mild (39 degrees C to 42.5 degrees C) hyperthermia with continuous irradiation, as well as the effects of short (45 degrees C to 46 degrees C) heat treatments followed by continuous irradiation at various temperatures will be studied.

134. DEOXYNUCLEOSIDE TRIPHOSPHATE POOLS, CANCER CHEMOTHERAPEUTIC AGENTS AND SPONTANEOUS MUTATION IN CULTURED MAMMALIAN CELLS (ABBREV)

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The objectives of our research are: 1) to determine the role of the pools of deoxynucleoside triphosphates in maintenance of the fidelity of DNA replication and mutagenesis in cultured mammalian cells, and 2) to examine the effects of cancer chemotherapeutic agents on these pools.

Our approach to this problem has been to isolate mutants of cultured Chinese hamster ovary cells (CHO) with altered pools of deoxynucleoside triphosphates and then to determine the effect of these alterations on defined genetic markers. We have recently isolated a class of mutants of CHO (called thy negative) by a single step selection for resistance to arabinosyl cytosine (araC). This mutant has altered pools of pyrimidine deoxynucleoside triphosphates - it has a 5- to 10-fold expanded pool of dCTP but is unable to synthesize dTTP and is thus dependent upon an exogenous supply of thymidine for maintenance of the pool of dTTP.

As a pleiotropic effect of the thy negative mutation, the rate of spontaneous mutation in thy negative cell lines is increased 5- to 200-fold for several genetic markers. Currently we are examining these mutations in detail to determine if thy negative affects the fidelity of DNA replication as a result of 1) base mispairing caused by the altered triphosphate pools; 2) induction of an error-prone repair mechanism or 3) disturbing cellular proofreading mechanisms. We also intend to examine the relevance of our results obtained with this model cell system to in vivo araC chemotherapy. White blood cells from patients relapsing after araC treatment will be examined for deoxynucleoside triphosphate content to determine if the resistance of these patients to araC is the consequence of alterations of the triphosphate pools. Our results suggest that such alterations of triphosphates have significant consequences for the success of further drug treatments.

135. THE USE OF CYCLIC NUCLEOTIDE ANALOGS IN THE CHEMOTHERAPY AND RADIOTHERAPY OF TUMORS

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OBJECTIVE: Study of the mechanism(s) of action of cyclic nucleotide derivatives on growth of cultured hepatoma cells.

APPROACH: Cell cycle and pedigree studies of rat hepatoma cells cultured in the presence of cyclic nucleotide analogs either with or without previous X-irradiation.

B. ANALYSIS OF TRANSFORMATION IN CELL CULTURES

136. NEW CELL SYSTEMS FOR THE STUDY OF THE BIOLOGY OF MUTATION AND NEOPLASTIC TRANSFORMATION

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Cultured mammalian cells have many advantages in screening for biological activity in the effluents and by-products of

various energy technologies. To begin with, they are closer to mammals than are bacteria, and they are appreciably more economical in time and cost than animal systems. Also, mammalian cells may be used to pursue studies of mechanisms at levels not possible with animals. As cells from different species become available, they may be used to develop extrapolative procedures from mouse to man. Cells of mesodermal origin, specifically fibroblasts, are at present the cell type primarily available for screening purposes. However, because there are only relatively few mesodermal cell lines available for studies of this type, additional cell systems should be established to insure that generalities may be extracted from the collective results. Furthermore, epithelial cell systems are needed for the assessment of mutation and neoplastic transformation since most human cancers originate in epithelium. This program is intended: (1) to establish epithelial- and additional fibroblast-derived cell lines for the measurement of mutation and neoplastic transformation; and (2) to examine the biological mechanisms responsible for expression time after transformation and its relationship to expression time after mutation induction.

BIBLIOGRAPHIC REFERENCES: M.M. Elkin and A. Han, Neoplastic Transformation and Dose Fractionation: Does Repair of Damage Play a Role, Radiat. Res. 79: 233-240 (1979).

137. A NEW ASSAY FOR TRANSFORMED CELLS

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A new assay for transformed cells is being developed using as the marker plasminogen activator. The basis of the assay is a new fluorogenic substrate which is an active site titrant of plasmin. This derivative of fluorescein is at least 20-fold more sensitive than presently available derivatives. The derivative has been shown to be an excellent active site titrant of trypsin, plasmin, thrombin, urokinase, factor Xa, factor IXa and has been used to characterize the activation of plasminogen by urokinase. To measure the plasminogen activator of transformed cells, 10,000 cells are incubated in microwells with purified plasminogen. The resultant plasmin is then titrated with the derivative. The assay is quick, sensitive, and quantitative. As few as 250 transformed cells can be assayed. The Km(app) and Vmax for the activation of plasminogen by transformed cells, in situ, can be determined. This assay is now being used to study the correlation between plasminogen activator activity and tumorigenicity.

138. ANALYSIS OF GENE CONTROLLED EVENTS IN NEOPLASTIC TRANSFORMATION

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Sexual and parasexual genetic analysis has permitted the detection, characterization and genetic localization of a number of mammalian cellular genes which participate in endogenous type C virus expression. Six classes of genes are under study: (1) endogenous cellular DNA sequences homologous to type C viral RNA; (2) integration sites of retroviruses in mammalian chromosomes; (3) viral cell surface receptors; (4) restriction genes which delimit viral replication; (5) enzyme structural genes; (6) cell surface antigens including MHC. Examples of each of these gene classes have been detected and mapped in human and feline model systems. The allozyme genetic signature as a definitive characteristic of cultured cells has been applied to monitors of cell contamination, quality control of in vitro transformation and identification of tissue origin in human embryonic development. Allozyme typing and karyologic analysis have demonstrated the triploid and androgenetic origin of partial and complete hydatidiform moles respectively. Population genetic analysis of human feline, and murine populations has revealed evolutionary conservation of linkage associations and the characters of genetic variance.

BIBLIOGRAPHIC REFERENCES: Rice, M.C., and O'Brien, S.J.: Genetic variance of laboratory outbred Swiss mice. Nature 283:157-167, 1980. Gardner, M.B., Rasheed, S., Pal, B.K., Estes, J.D. and O'Brien, S.J.: Akvr-l, a dominant MuLV restriction gene is polymorphic in leukemia-prone wild mice. Proc. Natl Acad. Sci. 77:531-535, 1980.

139. BIOCHEMISTRY AND CELL CULTURE RESOURCE

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OBJECTIVES: Culture of mammalian cells including human epithelial cells. Invitro transformation assays with mammalian cells. Cell- and tissue-mediated mammalian cell mutagenesis and transformation assays. Measurement of carcinogen-modified macromolecules by biochemical and immunological assays. Preparation of specialized tissue culture media and related reagents. Chemical and enzymatic synthesis of derivatives of vitamin A.

140. CHOLESTEROL IN MEMBRANE FUNCTION AND CELL TRANSFORMATION

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Substantial evidences have shown that cholesterol synthesis is an important metabolic process which is intimately related to the control of cellular proliferation and expression of several membrane associated functions. However the mechanism through which cholesterol synthesis is regulated is not fully understood. It is generally accepted that the rate-limiting step in the sterol synthetic pathway is the generation of mevalonate, an intermediate metabolite catalized by 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase. We reason, therefore, that investigation of the mechanism by which the activity of HMG-CoA reductase is regulated is necessary to allow us to understand not only the control of sterol synthesis but also the regulation of cell growth and proliferation. Several oxygenated derivatives of cholesterol, e.g., 25-hydroxycholesterol, specifically inhibit the activity of HMG-CoA reductase either by repression of the enzyme synthesis or by acceleration of the enzyme degradation. Several mevalonate-like fungal metabolites, e.g., compactin, competitively inhibit the activity of HMG-CoA reductase when they were incubated with cell cultures. Upon removal of these metabolites by washing the level of HMG-CoA reductase was found to increase by a still unknown mechanism. The objective of the present research project is to investigate the mechanisms by which oxygenated sterols, mevalonate-like fungal metabolites and other metabolic inhibitors affect the activity of HMG-CoA reductase.

141. HUMAN CHROMOSOMES IN HUMAN CANCER CELLS

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This is part of a broader project. A summary of this subproject is not available.

142. DEVELOPMENT OF A MODEL SYSTEM FOR IN VITRO TRANSFORMATION - MAPPING OF THE XERODERMA PIGMENTOSUM GENES

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Xeroderma pigmentosum (XP) complementation group A, C, and D genes will be mapped in mouse, chimpanzee, and human genomes. The purpose of mapping these genes is to 1) identify genetic components of the DNA repair systems in the mouse, and 2) facilitate the use of XP lines in future studies on the genetic analysis of neoplastic transformation. SV40 transformed XP lines will be used as recipient cells in fusions with microcells prepared from appropriate donors. Hybrids containing the complementing chromosome will be selected by resistance to killing following UV irradiation. Donor chromosomes carrying the dominant, wild type XP alleles will be identified in the hybrids by karyotypic analysis. Location of the XP genes in the human complement will be aided by prior mapping in the chimpanzee. Human lines carrying translocations and/or isozyme markers on chromosomes homologous to mapped sites in chimpanzee will be used as microcell donors in human XP-human microcell fusions.

143. DEVELOPMENT OF ASSAY SYSTEM FOR IN VITRO TRANSFORMATION BY MURINE LEUKEMIA VIRUSES

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Using colony stimulating factors, culture techniques for long-term culture of normal hematopoietic cells have been established. Nontumorigenic bone marrow cells from NIH/Swiss, 129/J and C57BL/6 mice and spleen cells from NIH-Swiss, CBA and C57BL/6 mice have been maintained for over 12 months. Morphological and histochemical studies revealed that these cells are metachromatic cells capable of differentiation to basophilic granulocytes. These cells contain receptors for mouse IgE and histamine, which can be used as differentiation markers. Although these cells did not show colonies in spleens of lethally irradiated mice, colony and cluster formation was observed in methylcellulose medium, indicating that committed stem cells are maintained in suspension culture. These cell lines may be useful in studying the relationship between cell differentiation and viral oncogenesis.

144. REGULATION OF EXPRESSION OF VIRAL TRANSFORMATION

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Chemical and viral transformants of the established mouse cell line 3T3 have been identified which behave in a 'dominant' manner in cell hybrids with untransformed 3T3. These cells are being utilized in gene transfer experiments with normal human fibroblasts employing techniques of microcell fusion and DNA transformation in an effort to 'map' and isolate genetic loci responsible for expression of transformation.

BIBLIOGRAPHIC REFERENCES: Jha, Cacciapuoti and Ozer. Expression of Transformation in cell Hybrids: II. Non-suppression of the transformed phenotype in hybrids between a chemically transformed and non-transformed derivatives of BALB/3T3 J. Cell Physiol. 97: 147-152, 1978.

145. NORMAL AND TUMOR-VIRUS TRANSFORMED CELL BEHAVIOR

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The objectives of this proposal are to determine which cellular components regulate the motility and behavior of cultured normal cells and their transformed derivatives. Emphasis is placed on the function of the major components of the system of cytoplasmic fibers present in the majority of mammalian cell types including microfilaments, 10 nm filaments and microtubules. These cytoplasmic fibers are thought to be responsible for controlling many aspects of behavior including cell attachment, spreading, surface topology, shape formation, locomotion, division and contact inhibition. The long-term goal of these studies is to gain new insight into the mechanisms involved in the cellular basis of transformation to the neoplastic state which ultimately results in malignant tumor formation and metastasis. Experimental approaches involved in relating structure and function in this proposal include microscopical, physiological, immunological and biochemical techniques.

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146. STUDIES ON INITIATION AND PROMOTION OF CELL TRANSFORMATION

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OBJECTIVES: Studies on the effects of carcinogens on the induction of mutation and cell transformation in mammalian cells in vitro. Studies on the effect of DNA-repair on induced frequencies of mutation and cell transformation. Studies on the effects of tumor promoters on the induction of somatic crossing-over mutation and cell transformation.

APPROACH: Simultaneous determination of mutation frequencies and cell transformation frequencies in the same cell line. Comparison of mutation induction and cell transformation induction before and after liquid holding. Use of a heterozygous cell line for the detection of somatic crossing-over.

147. GROWTH FACTORS AND RECEPTORS IN CHEMICAL TRANSFORMATION

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The specific alterations which occur in the control of cell proliferation in chemically transformed cells will be explored in this project. Data obtained in this laboratory using the nontransformed AKR-2B and C3H/10T1/2 mouse embryo derived cell lines and the chemically transformed derivatives of these cells, called AKR-MCA and C3H/MCA-58, respectively, have indicated that most of the characteristics of the transformed cells could be accounted for by continuous mitogenic stimulation with a growth factor. Both of the nontransformed cells have epidermal growth factor (EGF) receptors and are stimulated to proliferate by EGF. The transformed cells are not stimulated by EGF and have no detectable EGF receptors. Preliminary studies indicate that the AKR-MCA cells secrete a factor (or factors) into serum free medium which stimulates cell proliferation in the resting nontransformed AKR-2B cells and which competes for EGF binding on these cells. The studies in this project are designed to test the hypothesis that autostimulation of cells by growth factor which they themselves produce may be one mechanism in the chemical transformation of cells. This will be accomplished through the identification and attempted purification of growth factor-like substances produced by the chemically transformed cells (AKR-MCA and C3H/MCA-58) and not by the nontransformed cells (AKR-2B and C3H/10T1/2). Partially purified or 'purified' growth factor will be charcterized with respect to its biological and chemical properties. Changes in the EGF receptors of nontransformed cells at various stages of the cell cycle and with continuous mitogenic stimulation with growth factors that do not compete for this receptor will be examined. It will also be determined whether the inability of the chemically transformed cells to bind 125I-EGF is due to occupancy of the receptor by a secreted growth factor, masking of the receptor by other cell constituents, or rapid inactivation (down regulation) of receptor. The proposed experiments should yield data valuable in proving or disproving the hypothesis.

BIBLIOGRAPHIC REFERENCES: Robinson, R.A., Ryan, R.J., and Moses, H.L. Cell cycle and chemical transformation effects on epidermal growth factor receptors. J. Supramol. Struct., Suppl. 4:168, 1980. Moses, H.L. and Robinson, R.A. Growth factor, growth factor receptors and cell cycle control mechanisms in chemically transformed cells (Symposium). Fed. Proc. In press, 1980.

148. GENETIC ANALYSIS OF MALIGNANT TRANSFORMATION

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Malignant transformation induced by chemical carcinogens is a stable heritable trait, yet neither the mechanism by which it is induced nor the character of the trait once it is expressed is known with certainty. It has been suggested that chemicals may induce transformation by causing either a somatic gene mutation or an epigenetic change in gene expression. Once induced, the transformation may be maintained either by the continued expression of some dominant transforming function or by the recessive loss of a function necessary to keep the cell normal. A genetic analysis of both the induction and expression of chemically induced transformation is planned to differentiate between these sets of alternatives. Correlations between mutation and transformation induced in vitro by chemical carcinogens will be sought to test the somatic mutation hypothesis in rodent and

human cells. Chemically transformed clones will be analyzed by somatic cell hybridization to determine if transformation is a dominant or recessive trait and to obtain an estimate of the number of genes involved.

BIBLIOGRAPHIC REFERENCES: Bouck, N., and di Mayorca, G., 1979. Evaluation of chemical carcinogenicity by in vitro neoplastic transformation. in Methods in Enzymology, W.R. Jacoby and I. Pastan, eds. 58:296-302. Pintel, D., Bouck, N., di Mayorca, G., Thimmappaya, B., Swerdlow, B., and Shenk, T., 1979. Simian virus 40 mutant ts A 1499 is heat-sensitive for lytic growth but generates cold-sensitive rat cell transformants. Cold Spring Harbor Symposium in Quant. Biol XLVI.

C. GENETIC STUDIES OF CULTURED MALIGNANT CELLS

149. CHROMOSOME ANALYSIS OF HUMAN BLADDER CELL LINES

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A previous report (ICRF Scientific Report 1978 - report 155) described: 1. the ploidy of 5 human bladder carcinoma cell lines; 2. the karyotype of 4 of these lines, EJ, J82, T24 and RT112; and 3. the cloning of the EJ cell line.

The karyotype of the fifth cell line, RT4, shows none of the marker chromosomes seen in the other 4 cell lines. No marker chromosome is common to all 5 cell lines, but there is one normal haploid set of chromosomes present, including the sex chromosomes. Many of the other normal chromosomes are either lost or duplicated but no particular chromosome is consistently involved.

All 3 anaplastic cell lines, EJ, J82 and T24, grow in agar but only EJ and RT112 produced tumors in nude mice. This lack of correlation between the growth in agar and tumorigenicity agrees with those findings of Marshall et al., (Journal of the National Cancer Institute 1977, 58, 1743). However, the three marker chromosomes, M1, M2 and M3, found in the anaplastic cell lines seem to be associated with the expression of growth in agar.

Cloning of the EJ cell line at early passage gave both diploid (beta, 5E and 8D) and tetraploid (2B, 3D, 3E and 9F) clones. The three diploid clones all retained markers, M1, M2 and M3, but varied in the other marker chromosomes present. Clone 8D has a Philadelphia (Ph) chromosome. All 7 clones grow in agar but 2 of these were non-tumorigenic (5E and 3E).

Further analysis with these cell lines and clones may help to explain the relationship between the chromosome constitution of these cells and the expression of a transformed phenotype.

150. CYTOGENETICAL ANALYSIS OF MALIGNANT HUMAN TUMORS IN VIVO AND IN VITRO

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OBJECTIVES: To examine karyotypes from malignant human effusions to diagnose malignancy and to assess chromosome evolution in cells from these effusions at various passages in vitro.

APPROACH: Specimens from pleural, pericardiac and ascitic effusions from patients at the University Hospital in Lund (Sweden) are incubated with colchicine for at least 1 hour, treated with a hypotonic solution and fixed in Carnoy's fixative before staining. The presence of abnormal metaphases with specific chromosome stemlines indicates malignancy and cytogenetical analysis has proven to be a valuable diagnostic tool, especially when standard cytological methods fail to give a definite diagnosis. Following staining with chromosome banding-techniques and detailed karyotype analysis of the in vivo slides, cells from the malignant exudates are inoculated into plastic culture flasks and established in vitro as cultures. The cultures are cloned, injected into nude mice of NMRI genetic background and the tumor nodules growing subcutaneously in the nude mice are minced and again established as cell cultures.

Chromosome analyses are then performed at various passages in vitro, with G-, C- and N-banded chromosomes and in some cases even harlequin-stained chromosomes. The frequency of marker chromosomes and double minute-chromosomes as well as the distribution of chromosomes are assessed and karyotype evolution examined under varying environmental conditions, with different nutrient media etc.

PROGRESS: Preliminary results indicate that specific marker chromosomes can be found in many malignant human cells. These markers vary from tumor to tumor, but can be relatively consistent within the same tumor. The value of cytogenetical analysis as a diagnostic tool has been well established.

BIBLIOGRAPHIC REFERENCES: Korsgaard, R. and Mandahl, N.: Chromosome studies of tumours and tumour cell lines. (In Swedish) Cancer-Journal of the Swedish Cancer Society 1:19, 1978.

151. ANALYSIS OF DNA REPLICATION AND SISTER CHROMATID EXCHANGES IN METAPHASE CHROMOSOMES USING BRDU-33258 HOECHST TECHNIQUES

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Chromosome replication and the response of mammalian cells to clastogenic agents will be studied by photomicroscopy. BrdU-33258 Hoechst fluorescence techniques will be used to study DNA replication kinetics in highly extended human chromosome preparations. Resolution of replication kinetics in human and hamster cells will be extended to the level of specific sequences, using restriction enzyme digestion, electrophoresis, and hybridization methods. Flow sorting of viable cells in narrow S phase windows will be combined with cytological techniques to estimate the intercellular fluctuation in the internal order of DNA replication. Flow cytometry and BrdU dye methods will also be used to assess the cell cycle dependence of SCE induction by different clastogens. The ability of the tumor promoter, TPA, to induce SCEs or enhance SCE induction will be compared with effects on cellular proliferation and invasiveness in vivo, in the Chinese hamster cheek pouch. The ability of clastogens to alter the proliferation, induce SCEs and mutations in cells from patients with Fanconi's anemia and Bloom's syndrome will be examined, in an attempt to determine the relationship between the chromosomal abnormalities and other pathological features of these diseases.

152. CELL GENETIC STUDIES OF LEUKEMIC DISORDERS

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OBJECTIVE: The aim of this project is to study the role played by the non-randomly acquired chromosomal abnormalities found in leukemic cells in the initiation and development of leukemia. The first target will be the Philadelphia anomaly: t(9q postive; 22q negative) characteristic of chronic myeloid leukemia (CML).

APPROACH: Three approaches are planned: a) Cytogenetic survey of bone marrow karyotypes of patients with chronic and acute leukemia and possibly preleukemic disorders, using banding techniques. Follow-up studies of the patients with acquired chromosomal abnormalities are designed to ascertain the clinical evolution: remission, relapse, acceleration, clonal evolution, therapeutic resistance, etc. b) Development of a specific clonogenic assay for leukemic cells which would permit in vitro studies of the biological characteristics of leukemic cells and their therapeutic sensitivity or resistance. c) Interspecies and intraspecies somatic cell hybridization between human leukemic cells and other cells. The role of individual chromosome (e.g., the Ph1 chromosome) in tumorigenicity will be studied (interspecies hybrids).

PROGRESS: 1. Survey of the patients in the region is organized and the follow-up study is set up. 2. A clonogenic assay for leukemic cells has been developped: the PHA-leucocyte feeder assay. 3. Hybrid clones were obtained by fusion between tumorigenic rodent cell lines and human normal leukocytes or CML cells.

Analyses in hybrid clones of the segregation patterns of the chromosomes involved in the Ph1 anomaly (9, 9q positive, 22, 22q negative) and the enzyme markers known to be located on

chromosomes 9 and 22 indicate that in different patients the breakpoint in the Ph1 chromosome (22q negative) might be different.

153. GENETIC STUDIES WITH CULTIVATED MAMMALIAN CELLS

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The human HPRT locus will be cloned. Radioactive cDNA probe will be made on template in RNA from HPRT-positive cells that does not associate with DNA from human cells that have an X-ray-induced deletion of the HPRT locus. The probe, nitrocellulose transfer techniques and autoradiography will be used to screen plaques formed by shotgun recombinant DNA phages for those with inserts complementary to probe sequences. After these clones are amplified those with functional HPRT genes will be identified by transfer of their DNA into HPRT-negative cells using microinjection or calcium-phosphate precipitation techniques.

If the approach outlined above is successful we will attempt to clone genes of the human major histocompatibility complex using DNA from cells having homozygous deletions in this region, which we have produced with x-rays.

Microinjection experiments and hybrids produced by fusing human malignant with non-malignant cells will be used to determine if the following traits are dominantly expressed: morphological transformation, anchorage-independent growth and tumorigenicity in nude mice. For traits that are dominantly expressed a combination of DNA-mediated genetic transformation and microinjection techniques will be used to clone the responsible cancer genes.

154. EXTRACHROMOSOMAL DNA IN MAMMALIAN CELLS

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OBJECTIVES: (1) To investigate the propagation, segregation and possible recombination of mitochondrial DNA (mtDNA) from different cell types when combined in a common cytoplasm; (2) to characterize mtDNA from cell lines carrying cytoplasmically transmissible markers.

APPROACH: We have previously isolated human and mouse cell lines resistant to chloramphenicol and have shown the trait to be cytoplasmically transmissible. We have also isolated a human cell line resistant to antimycin A. Preliminary observations suggest that this trait also is determined extrachromosomally. We intend to investigate further the properties of this cell line.

CAPR, ANTR, cell hybrids have been obtained and initial examination indicates that such hybrids are resistant to either antibiotic alone, but not to a combination of both. We intend to use both genetic and physical approaches to investigate the interaction between the parental type mtDNA molecules in these and other somatic cell hybrids.

mtDNA sequences from mouse and human sources have been cloned in bacteria and are being employed as probes to examine inter- and intra-specific variation in mtDNA sequences and to follow the fate of mtDNA after fusion of somatic cells.

155. DEOXYNUCLEOSIDE TRIPHOSPHATE POOLS, CANCER CHEMOTHERAPEUTIC AGENTS AND SPONTANEOUS MUTATION IN CULTURED MAMMALIAN CELLS (ABBREV)

Meuth, M. L., Institut de Recherche Clinique de Montreal, Laboratory of Molecular Genetics, *110 Ouest Ave. de Pins, Montreal, Quebec*, H2W 1R7, Canada

The summary for this project is under entry no. 134.

156. REPAIR OF COVALENT ADDUCTS FORMED BETWEEN BENZO(A)PYRENE (B(A)P) AND DNA BY MOUSE EMBRYO FIBROBLASTS

Frearson, P. M., Kirby, P., Thomson, R., Imperial Cancer Research Fund, DNA-Carcinogen Interactions Laboratory, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

Since most mammalian cells have evolved mechanisms for removing damage from their DNA we are investigating whether mouse fibroblast cells in tissue culture can excise B(a)P adducts from their DNA. Cells, labelled with 14C-thymidine while growing to confluence, are exposed to 3H-B(a)P for six hours; they are then maintained, for up to six days, in a resting state in medium containing 0.5 percent serum. When the DNA is extracted and degraded to nucleosides, the nucleosides containing 3H-B(a)P adducts can be separated on LH20-Sephadex columns, and the rate at which they are removed can be measured. Although approximately 60 percent of the adducts are removed within 72 hours, the remaining 40 percent are much more resistant to excision and these adducts are not removed when the cells double in number. We are attempting to determine the distribution of the repair-resistant adducts in the mouse DNA by the methods described in a previous report (ICRF Scientific Report 1978 -report 214) and to isolate from mouse embryos by methods already developed, (ICRF Scientific Report 1977 - reports 197 and 198) the proteins involved in the excision of the adducts. Similar methods are being used to study the binding to, and excision from, mouse embryo fibroblasts of compounds of the cyclopenta(a) phenanthrene series synthesized in the Chemical Laboratory. We are collaborating with the Cellular Pathology Laboratory to use baby mouse kidney cells grown in tissue culture to provide primary epithelial cells on a scale large enough to permit us to use our biochemical techniques.

157. DNA REPAIR AND RECOMBINATION (WITH T4 PHAGE) AND POLY ADP RIBOSYLATION (WITH MAMMALIAN CELL SYSTEMS)

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DNA repair and recombination.

OBJECTIVE: To design an in vitro system to study the molecular mechanisms of DNA repair and recombination; specifically, to study the function and properties of the gene products involved in these processes.

APPROACH: Since it is difficult to study DNA repair (under in vitro conditions), we are attempting to couple DNA repair to DNA replication. Since repair (and recombination) mutants are defective in DNA replication after DNA damage, we are attempting to devise conditions to complement with wild-type proteins.

Poly-ADP ribosylation.

OBJECTIVE: To study the role of poly-ADP ribose in the cell cycle.

APPROACH: A search and study of mutants involved in poly-ADP ribosylation. For this study we are currently using Chinese hamster ovary cells. We are also studying the biochemistry involving the poly-ADP ribose polymerase reaction.

158. POSTREPLICATION REPAIR IN CULTURED MAMMALIAN CELLS

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The objective of the proposal is to determine the molecular events involved in replication of a chemical carcinogen damaged DNA template in cultured mammalian cells treated with the proposed ultimate carcinogenic form of benzo(a)pyrene, 7,8 dihydroxy 9,10-epoxy-7,8,9,10-tetrahydrobenzo(a) pyrene, (B(a)P-diol-epoxide). The proposed studies make use of primary cultures of mouse epidermal cells and Vero cells (an African green monkey kidney cell line). Four specific objectives include 1) to continue to develop evidence for 'gapped DNA synthesis' in epidermal cells using DNA fiber autoradiography in order to study DNA fork progression and replicon function, 2) to determine if B(a)P-diol-epoxide DNA adducts in Vero cells act as absolute blocks to DNA replication and if not to determine the mechanism by which the whole genome is replicated, 3) to determine if B(a)P-diol-epoxide treatment of epidermal cells or Vero cells results in host cell reactivation of either UV-irradiated or B(a)P-diol-epoxide treated herpes simplex virus and whether reactivation is mutagenic to the virus and inducible, 4) to determine at the molecular level whether there is an enhanced rate of DNA elongation which might be related to B(a)P-diol-epoxide induced reactivation of the herpes simplex virus.

BIBLIOGRAPHIC REFERENCES: Bowden, G.T., and Fusenig, N.E., Caffeine Inhibition of Postreplication Repair of

UV-Damaged DNA in Mouse Epidermal Cells. Chem. Biol. Interact. (in press), 1980.

159. IN VITRO TRANSCRIPTION OF EUCARYOTIC GENES

Baldi, M. I., Gandini Attardi, D., Otsuka, A., Consiglio Nazionale delle Ricerche, Lab. of Cell Biology, *Rome, Italy*

OBJECTIVE: Mechanisms of expression of eucaryotic genes.

APPROACH: In vitro cell free systems.

PROGRESS: An RNAse activity, RNAse XLI, responsible for the excision of the intervening sequences (IVS's) from two yeast tRNA precursors, pre-tRNATyr and pre-tRNA3Leu, has been purified 54-fold from nuclear extracts of Xenopus laevis oocytes. As a preliminary step to the RNAse purification, the reaction products were characterized and a quantitative assay developed. Cleavage of pre-tRNA3Leu by RNAse XLI, and subsequent ligation of the half-tRNA molecules did not require prior processing to remove 5' leader or 3' trailer sequences.

The most completely purified RNAse XLI preparation cleaves tyrosyl tRNA, leucyl tRNA3 and tryptophan tRNA precursors.

The partially purified RNAse XLI was active over a broad pH range (from less than pH 5.5 to more than pH 8.0) and was optimally active at temperatures at or above 22 degrees C in buffers containing 150 mM salt (NH4Cl) and 4mM MgCl2. While MgCl2 and CaCl2 were able to satisfy the divalent ion requirement, 5 mM MnCl2 or ZnCl2 were unable to produce activity. RNAse XLI was inhibited by ATP, polyuridylic acid and total yeast RNA.

160. STUDIES ON THE CONTROL OF CELL DIVISION IN YEAST

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I am investigating the control of cell division, my specific goals being the identification of the genes involved, the elucidation of their molecular products, and ultimately the determination of the physiological roles that these play. Yeast (Saccharomyces cerevisiae), a simple eukaryotic organism amenable to both genetic and biochemical investigations, will be employed for the proposed research. In yeast, division is normally controlled in response to nutrient limitation and in preparation for conjugation. Both forms of control are integrated in the G1 interval at a step of the cell cycle known as start. Undoubtedly, many gene products are directly involved and elucidation of their individual roles would be a major step in clarifying how control of cell division in eukaryotes is organized. In order to identify the relevant genes, mutants which cannot complete the start step were sought. To date, I have isolated 40 independent mutations and assigned them to four unlinked complementation groups. The procedure to be used to identify the start gene product will be the molecular cloning of the start genes by transformation of mutant yeast cells, the use of cloned genes to isolate complementary mRNAs and the translation of these RNAs in vitro to identify the gene products. Once the start gene products are identified, antisera will be prepared against them to be used as polypeptide-specific immunological probes. Both the metabolism and intracellular locations of the start gene products will be investigated.

161. CYTOGENETIC ANALYSIS OF MALIGNANCY

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This project consists of the following parts: A. Chromosomes and malignancy - Continued cytogenetic studies of murine T- and non-T-cell leukemias: 1. How regular is the association between 15-trisomy and T-cell leukemias? 2. Cytogenetic mapping of the chromosome segments that are crucially important in the leukemia associated 15-duplication. 3. Does chromosome 15 derived from leukemia-susceptible and -resistant strains, resp., have the same chance to duplicate in the course of leukemia induction in the common F1 hybrids? 4. Malignancy suppression by somatic cell hybridization: Studies on the role of chromosome 15. 5. Continued cytogenetic studies on non-T-cell leukemias. B. Continued studies on in vivo host-tumor cell fusion: 1. Early events during the fusion process in vivo. 2. Establishment of hybrids between T-lymphomas and host cells in vivo. 3. Malignancy tests on 24-hour in vivo hybrids. 4. Continued morphological studies of lymphoma/host cell hybrids.

162. GENETIC STUDIES WITH MAMMALIAN CELLS

Herzenberg, L. A., Ledbetter, J. A., Malvehy, R. M., Parks, D. R., Stanford University, School of Medicine, Dept. of Genetics, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

OBJECTIVES: 1. To identify and characterize lymphocyte cell surface determinants, especially those which mark functional lymphocyte subpopulations, and to functionally characterize lymphocyte subpopulations carrying different surface markers. 2. To genetically map loci controlling lymphocyte surface determinants. 3. To fuse myeloma cells with normal antibody-producing cells, in order to obtain B cell hybrid culture lines which secrete large quantities of antibodies of interest or use. 3. To study the genetics, immune mechanisms and relationships between the occurrence of reticulum cell sarcomas and abnormalities in regulation of the immune response in SJL/J mice.

BIBLIOGRAPHIC REFERENCES: Leonore A. Herzenberg, Samuel J. Black and Leonard A. Herzenberg. 1980. Regulatory circuits and antibody responses. Eur. J. Immunol. 10:1-11. Jeffrey A. Ledbetter and Leonard A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunological Reviews 47:63-90.

D. BIOCHEMICAL CHARACTERIZATION OF CULTURED TRANSFORMED CELLS

1. DNA AND RNA SYNTHESIS

163. FURTHER STUDIES ON VASOPRESSIN STIMULATION OF DNA SYNTHESIS IN QUIESCENT SWISS 3T3 CELLS - INTERACTION WITH GROWTH-PROMOTING FACTORS

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The finding that an increase in Na ion entry mediates the effect of growth-promoting factors on the Na ion-K ion pump prompted us to ask whether substances (hormones, ionophores) that are known to promote Na ion influx in certain cell types can be mitogenic for quiescent cells. Pursuing this possibility, we found that vasopressins are potent mitogens for Swiss 3T3 cells.

The effect of vasopressin on DNA synthesis is strikingly potentiated by addition of insulin, EGF, and/or FDGF. The synergistic interaction between vasopressin and the growth-stimulating factors occurs in serum-free, synthetic medium. Insulin at low concentrations (20-60 ng/ml) potentiated the mitogenic effect of vasopressin. The synergism of insulin with vasopressin is more prominent at higher insulin concentrations.

In the presence of concentrations of FDGF which are not mitogenic, vasopressin elicits a maximal stimulation of DNA synthesis at a concentration of approximately 3 ng/ml. Similar dose responses for vasopressin are obtained when DNA synthesis is assessed in the presence of insulin, either by autoradiography of labelled nuclei or by incorporation of 3H thymidine into acid-insoluble material. In other experiments, we found that the mitogenic potency of arginine-vasopressin is similar to that of lysine-vasopressin. Oxytocin is also mitogenic for 3T3 cells in the presence of insulin, but the concentration needed to produce a half-maximal effect is approximately 2 micrograms/ml. Clearly, 3T3 cells display a striking discrimination between the peptides; vasopressin is about 1000-fold more potent than oxytocin in stimulating DNA synthesis in quiescent cultures of 3T3 cells. We are currently studying the effect of the neurohypophyseal nonapeptides on Na ion and K ion movements in 3T3 cells.

164. INTERACTION OF REACTIVE METABOLITES WITH DNA IN INTACT CELLS

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During their biotransformation a great number of chemicals are converted to reactive metabolites. By interacting with nuclear DNA these metabolites may give rise to mutations and/or predispose the cell to malignant transformation.

We have developed a method for measuring DNA damage and DNA repair in cultured human fibroblasts incubated with different metabolizing systems by kinetic studies of DNA strand breaks induced by chemicals. In the proposed project we plan to further develop the technique of studying DNA strand breaks in human fibroblasts and also in isolated liver cells from rat. We are going to use this technique to study the formation and transport of DNA damaging metabolites, as well as to study the relationship between activation, DNA binding, DNA repair and carcinogenicity of different DNA binding substances.

165. MECHANISM OF ARABINONUCLEOSIDE METABOLISM IN HUMAN CELLS IN VITRO

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The objective of this proposal is to identify the metabolic pathways required for conversion of the naturally occurring toxic purine nucleosides, adenosine (Ado), 2'-deoxyadenosine (dAdo) and that of their pharmacologically active arabinonucleoside analogs, 9-beta-D-arabinofuranosyladenine (ara-A) and its 2-fluoro derivative, F-ara-A, in cultured human leukemic lymphoblasts, termed CEM. The approach in these studies will be to isolate mutants from CEM cell cultures which have been selected for resistance to the toxic effect of either Ado or dAdo. The mutants resistant to Ado and dAdo will be tested for cross-resistance to the two selective drugs as well as to the arabinonucleosides to determine whether different metabolic pathways are required for activation of Ado and dAdo or the two analogs to their toxic metabolite.

The nature of the biochemical alterations that are responsible for the resistance of the CEM mutant lines to the various toxic nucleosides will also be determined. This will include determination of the kinase activity for phosphorylation of the various nucleosides in the wild-type and mutant CEM cells and the kinetic properties of the various enzymes with purine nucleosides. Completion of these studies will provide information on the relationship of various nucleoside kinases to purine ribo- and deoxyribonucleoside metabolism and their arabinonucleoside analogs in lymphoid cells. Ultimately information gained from this project should lead to more effective clinical use of these two chemotherapeutic agents.

166. STUDIES ON THE MODIFICATION AND METABOLISM OF DNA POLYMERASES IN NORMAL AND TRANSFORMED CELLS

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OBJECTIVE: The purpose of this project is to determine how DNA replication is regulated in normal and transformed cells. For this purpose, the metabolism of DNA polymerases will be investigated during the cell cycle and in the transformed cells, and answers will be sought to such questions as how each species of DNA polymerase is synthesized and involved in the DNA replication machinery, what kind of modification occurs on DNA polymerases, and with which factor DNA polymerases interact.

APPROACH: Antibody, a very useful tool for the purpose, will be prepared against each of DNA polymerases. First, a large quantity of highly purified preparation of DNA polymerase will be obtained. Antibody will be made by a conventional method when the highly pure preparation is obtained. If the highly pure preparation is not available, a hybridoma technique will be used to get a monoclonal antibody. Immunofluorescence method and immuno-electron microscope technique are useful in clarification of the subcellular distribution of DNA polymerase and its change. Immunoprecipitation will make it possible to identify not only an active DNA polymerase but also an inactive or modified enzyme protein.

PROGRESS: We have already established the method for the large scale purification of DNA polymerases beta and gamma from chick embryos and obtained antibodies against them.

167. DNA POLYMERASES IN NORMAL AND NEOPLASTIC HUMAN CELLS

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The fundamental goal of my research program is the elucidation of the mechanisms involved in the coordination and control of DNA replication, chromosome segregation and cell division. Since it is a fundamental attribute of all living cells that there be rigorous coordination of chromosome replication and segregation to ensure precise equipartition of the genome at cytokinesis, it seems clear that the study of the mechanisms involved in this coordination is central to our understanding of the regulation of normal and abnormal cell growth. The present proposal is concerned with the continuation of our ongoing program of purification and characterization of the DNA polymerases from the human malignant cell line, KB. We have identified three distinct DNA polymerase activities in KB cells, two of which are associated with nucleus, and the third with the cytoplasmic fraction. We have expended our major effort to date on rigorous purification and characterization of these activities, and at the present time two of the enzymes appear to be homogeneous. We believe that only by working with homogeneous and rigorously characterized polymerase molecules will it be possible to investigate meaningfully structural and functional interrelationships among them and begin to explore how the regulation of their biosynthesis and intracellular distribution are related to the coordination and control of DNA synthesis in human cells. Morever, such studies provide an essential foundation for the understanding of alterations in DNA polymerase structure, function and intracellular distribution that may be associated with virus infection and transformation and with those alterations of normal cell growth regulation that are observed in hyperplastic and neoplastic states.

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168. BIOCHEMISTRY OF LATE MITOTIC EVENTS IN CULTURED CELLS

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The long-range goal of this research is to gain more understanding of the extensive structural rearrangements and alterations in macromolecular synthesis that occur during mitosis in cultured human cells. With this in mind, we propose to investigate specifically aspects of nuclear reformation and cytoplasmic reutilization of mRNA in the metaphase-to-G1 transition. For these studies, a cultured human cell line, HeLa S3, is being used to explore this period in the cell cycle because large numbers of cells homogeneous for metaphase, which by incubation enter G1, are obtained without the immediate use of inhibitors. In particular nuclear reformation will be studied by the use of phospholipid precursors to probe nuclear envelope reformation and by use of a defined nuclear subfraction, the nuclear matrix, of probe reformation of other nuclear structures. With regard to phospholipid metabolism, lipid precursors are incorporated as mitotic cells complete division even in the absence of renewed protein synthesis, and this incorporation can be visualized by electron microscope autoradiography at least by early telophase. We shall continue to select candidate polypeptides that coisolate with the nuclear matrix fraction in order to determine their topographical relationships and fate during late mitosis. Our recent information indicates that the disaggregation of polyribosomes during mitosis results in the release of free mRNP particles free of ribosomes. Only a portion of the total free metaphase mRNP particles reappear in progeny G1 cell polyribosomes. This now permits additional analyses to determine

whether or not there is a structural basis for reutilization of mRNP particles in G1 and conceivably, whether or not there is selective reutilization of mRNA.

It is not clear which events are either obligatory for or merely temporally coincident with cell division. This type of information should not only help to sort this out but, more important, conceivably could shed light on regulatory events involved in or associated with mitosis.

2. AMINO ACID AND PROTEIN METABOLISM

169. PRODUCTION OF ALKALINE PHOSPHATASE ISOENZYMES BY HUMAN MALIGNANT CELLS IN CULTURE

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The objectives of this project are to examine the alkaline phosphatase isoenzymes produced by tumor cells in culture and compare them to normal tissue-specific isoenzymes of alkaline phosphatase. Differences in either the polypeptide or carbohydrate portions of these enzymes could be exploited in the development of more specific tumor markers, and could prove useful in comparing glycoprotein synthesis and processing in normal and malignant cells. Tumor cell alkaline phosphatases are being compared to their normal counterparts by differential inhibition with noncompetitive stereospecific inhibitors, heat and urea stability, immunoreactivity, chromatographic behavior, and electrophoretic migration under nondenaturing conditions. In addition, the isoenzymes can be radiolabeled in vitro with phosphorous-33, or in vivo with radioactive amino acids or sugars. Such radiolabeled products are being analyzed by SDS-polyacrylamide gel electrophoresis. Several tumor cell lines were found to produce isoenzymes similar in many respects to term placental alkaline phosphatase. Peptide mapping studies showed that the polypeptide portions of these enzymes were very closely related. However, in many cases this tumor cell enzyme differed significantly from the placental form in catalytic properties, size, or degree of glycosylation. These data suggest that differences in the post-translational processing of the placental alkaline phosphatase polypeptide in tumor cells may result in altered catalytic or biochemical properties of this enzyme. The applicability of these differences to the development of tumor-specific alkaline phosphatase isoenzyme assays, as well as the molecular control mechanisms involved in the generation of these differences, are currently being investigated.

170. COLLAGEN BIOSYNTHESIS BY CULTURED HUMAN FIBROBLASTS

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In this grant application we are proposing extensive studies of the metabolism of collagen and its biosynthetic precursor, procollagen, by cultured human skin fibroblasts in control and disease states. The major goal of this research is to exactly define the molecular defects which may occur in the biochemistry of collagen in diseases affecting the connective tissues of skin and other organs. The major emphasis will be on diseases in which previous investigations by us and others have revealed connective tissue abnormalities, but in which the exact nature and the exact extent of collagen aberrations are currently unknown. Such diseases include psoriasis, benign and malignant skin tumors, scleroderma, the Ehlers-Danlos syndrome, and osteogenesis imperfecta. For comparison, parallel studies will be performed employing control fibroblasts from subjects without connective tissue abnormalities. The parameters to be studied include rate of procollagen synthesis by the culture fibroblast, as well as isolation, identification, and characterization of the genetically distinct procollagen. In order to understand changes in collagen metabolism in the fibroblast cultures in diseases, additional studies will be performed on the structure and metabolism of genetically distinct procollagens synthesized by normal human skin fibroblasts. Specifically, the procollagens will be isolated in quantitative amounts in order to allow further chemical characterization. The purification of the procollagens will also enable us to develop radioimmunoassays, based on type-specific antibodies, which will then be utilized in studies of collagen formation in diseases. The antibodies are also employed in investigations concerning the control mechanisms involved in the biosynthesis of procollagen as well as in identification of products in a cell-free translation system to be developed for assay of procollagen messenger-RNA.

171. THE ROLE OF PROTEIN PHOSPHORYLATION IN THE REGULATION OF DIFFERENTIATED FUNCTIONS OF CELLS IN CULTURE

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The proposed studies are designed to further our understanding of the role of protein phosphorylation in the regulation of certain differentiated functions of eukaryotic cells. They are based on the premise that information pertaining to the fundamental regulation of differentiated functions will be applicable to the study of pathological states. The plan is to study (a) the role of protein phosphorylation in the biochemical actions of insulin and related growth factors in cultured adipocytelike cells and (b) the role of cyclic (AMP-mediated and Ca ion-calmodulin-mediated) protein phosphorylation in regulating phagocytosis, motility and secretion in cultured macrophagelike cells. Our approach is two-pronged: (a) to define the relevant protein kinases and their substrates and (b) to examine function and regulation in cultured cells whose physiology and susceptibility to genetic manipulation make them especially suited to these kinds of investigations. The ultimate goal is to be able to use this information to augment, inhibit or otherwise regulate these biochemical processes so that physiological consequences can be pharmacologically controlled. This long-range plan will of necessity require the integration of a number of approaches including (a) analysis of the physiological and biochemical pathways involved in the particular protein phosphorylation reactions: the protein kinases, phosphoprotein phosphatases, their activators, inhibitors and substrates; and (b) the design of drugs that will specifically alter some step in the protein modification reactions. Our present program is concerned primarily with (a).

172. VIRAL INTERACTION OF MICROTUBULES IN CULTURED CELLS

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We have characterized HeLa cell microtubules obtained after 4 cycles of polymerization-depolymerization and have found a major accessory protein (MAP) associated with these microtubules which has a MW equal to 201-206,000 by SDS-PAGE. Further, by in vitro reconstitution experiments this MAP promotes assembly of HeLa cell tubulin to form microtubules at least as efficiently as calf brain MAP-2. Current experiments are aimed at determining if HeLa MAPs are essential for adenovirus edge-binding in vitro to microtubules. We had previously shown there was a need for brain MAP-2s in this assay. On another front we have performed a quantitative study of adenovirus binding to HeLa cell microtubules in vivo. We showed that after infection with adenovirus type 2 or 5, at 1 hour after infection, a significant portion of the virus particles observed (31%) was found to be associated with microtubules. On a random basis we would only have expected a 1% or 2% association of virus with microtubules. Currently, double immunofluorescence experiments are being performed to determine whether this early adenovirus association with microtubules can be visualized using antisera to tubulin in conjunction with antisera to virus.

BIBLIOGRAPHIC REFERENCES: Babiss, L. E., Luftig, R. B., Weatherbee, J. A., Weihing, R. R., Ray, U. R., Fields, B. N. (1979). Reovirus serotypes 1 and 3 differ in their in vitro association with microtubules. J. Virol. 30, 863-874. Weatherbee, J. A., Luftig, R. B., and Weihing, R. R. (1979). In vitro phosphorylation of the microtubule-associated proteins of HeLa cells. J. Cell. Biol. 83, 342a.

173. REGULATION OF MAMMALIAN PROTEIN BIOSYNTHESIS

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OBJECTIVE: The aim of the project is to investigate several aspects of the regulation of protein synthesis in eukaryotic cells. It is proposed to analyze in depth the mechanisms which are involved in control of translation at the cytoplasmic level in normal animal tissues and tumor cells grown in tissue culture. The modes of action of agents which affect cellular growth and division, including interferon and nutritional factors, will be studied.

APPROACH: The work will involve the preparation of rabbit reticulocytes and the growth of mammalian tumor cells in tissue culture. Cell-free extracts will also be prepared from these materials. Such systems will be used to study the mechanisms of action of interferon as both an anti-viral and an anti-cellular (anti-tumor) agent, and also the regulation of polypeptide chain initiation by variations in nutrient supply. The mode of action of haem in controlling protein synthesis in reticulocytes will also be investigated further.

Two complementary approaches will be used, as appropriate. In the first, attempts will be made to correlate the behavior of the intact cells with properties of the crude extracts derived from them. In the second, individual components of the protein synthesizing machinery (such as initiation factors and mRNA) will be purified and used to reconstitute in vitro systems for assays of partial reactions of protein synthesis.

Other aspects of the control of gene expression and cellular growth will also be studied in attempts to define the detailed mechanisms of action of interferon and of essential nutrient supply; DNA synthesis, RNA synthesis and the transmembrane transport of the precursors for macromolecular synthesis will be investigated in their appropriate contexts.

PROGRESS: The results which constitute the basis of this project show that protein synthesis in mammalian cells is regulated at the translational level primarily at the stage of polypeptide chain initiation. Control of cellular growth rates by variations in nutrient supply (e.g. essential amino acids), and of protein synthesis in reticulocytes by haem, both involve modulation of formation of (40S ribosomal subunit, Methionyl-tRNA) initiation complexes. (Text Abridged.)

174. FUNCTION OF SPECIFIC NUCLEAR PROTEINS IN THE CONTROL OF CELL PROLIFERATION

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OBJECTIVE: The purpose of this investigation is to elucidate the role of two specific nuclear proteins in the control of proliferation of eukaryotic cells in culture.

APPROACH: It has been shown previously that serum stimulation of quiescent mouse 3T3 cells induces a general increase of 3-4 times in the rate of biosynthesis of nuclear proteins. Two nuclear proteins respond dramatically and specifically to growth stimulation: the synthesis of the second, P2, is coincident in time with the duplication of the chromatin. The polypeptide P2 will be purified and characterized. Antibodies will be raised to the polypeptide and the sub-nuclear localization examined. The function of P2 in nuclear physiology will be investigated by establishing in vitro systems for RNA transcription and DNA replication. The effect of addition of exogenous P2 or anti-P2 will be analyzed. We will also examine the importance of post-translational modification in this context. The problem of the function of these polypeptides will also be approached by examining their response to different types of restimulation, i.e., starvation and readdition of an amino acid and temperature shift of temperature-sensitive G1 mutants.

175. NUCLEAR PROTEIN METABOLISM IN NEOPLASIA

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Research is proposed to study the synthesis and phosphorylation of H1 histones in tissue culture cells, somatic cell hybrids and organ cultures of normal and malignant mouse mammary tissues exposed to different hormones. Chromatographic resolution of H1 histone subtypes will be used to assess hormonal effects on the synthesis of various H1 subfractions in organ cultures, while tryptic phosphopeptide maps will be used to detect cell- and cancer-specific effects on the phosphorylation of H1 histone subtypes in various cells. A model for differential H1 histone gene expression in mouse-mouse and mouse-rat somatic cell hybrids will be constructed based on the chromosome contents and ratios in numerous hybrid clones. Chemical crosslinking and nuclease digestion will be used to probe the topography of chromatin and discern specific spatial relationships among H1 histone subtypes and their phosphorylated forms.

176. INVESTIGATION OF THE GENETICS OF AMINO ACID METABOLISM AND MEMBRANE STRUCTURE AND FUNCTION IN CULTURED MAMMALIAN CELLS

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OBJECTIVE: Genetic analysis of arginine metabolism.

APPROACH: Techniques are being developed for the isolation of mutants of cultured mammalian cells and their subsequent genetic and biochemical analyses. Present approaches concentrate on the isolation of cells resistant to toxic analogues of arginine, and of those displaying a growth requirement for arginine. The technique of interspecies cell fusion is being used to assign genes involved in arginine metabolism to human chromosomes and to specific subregions of them.

PROGRESS: Mouse teratocarcinoma cells resistant to the toxic arginine analogue canavanine have been obtained. They show a reduced incorporation of radiolabelled arginine into protein, the basis of which is under investigation. The human gene for argininosuccinate synthetase has been assigned to chromosome 9 (Exp. Cell Res., 106, 71 (1977)). With the use of translocation mapping it has been further localized to the distal one-fifth of the long arm of this chromosome.

3. BIOSYNTHESIS AND SECRETION OF HORMONES

177. ISOLATION, CHARACTERIZATION AND DEVELOPMENT OF ASSAYS SPECIFIC TO THE MAJOR FORMS OF PEPTIDE HORMONES IN CANCER PATIENTS

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The major objectives are to identify the immunoreactive forms of peptide hormones present in the sera of cancer patients and to study their biosynthesis and secretion in cloned cell lines of human origin. Our data indicate that the major forms of calcitonin in cancer patients' sera exhibit molecular weights greater than or equal to 30,000 and that the calcitonin monomer represents only a minor constituent of the total immunoreactive calcitonin. Our results indicate, however, that certain forms of immunoreactive calcitonin may result from aggregation or binding to other serum proteins. Human cloned cell lines that produce the major forms of calcitonin observed in cancer sera have been developed. Currently, we are isolating these forms of calcitonin from tumors developed in nude mice in order both to characterize them biochemically and to develop radioimmunoassays specific for their detection.

178. APPLICATION OF THE ARTIFICIAL CAPILLARY CULTURE TECHNIQUE TO POLYPEPTIDE HORMONE PRODUCTION

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The artificial capillary culture technique provides a pericellular microenvironment that closely resembles the in vivo state. This may allow normal cells to maintain their differentiated functions for prolonged periods of time in vitro. Cells are maintained in a physiologic state without many of the artifactual conditions imposed by more standard methods of tissue culture.

The technique is, therefore, especially well suited for the culture both of cells of endocrine origin and of their target cells. Response of cells to various hormones and their culture over long periods have been achieved, thus making the techniques useful for continuous production of hormones for both laboratory and clinical uses. Modifications have been made to the basic design. These permit study of nutrient and product transport through the tissues formed in the intercapillary spaces, the effect of lymphatic-type drainage upon cell function, and effects of a physiologic collagen sub-stratum upon cell growth.

BIBLIOGRAPHIC REFERENCES: Knazek, R.A., Gullino, P.M., and Frankel, D.S. Dual circuit woven artificial capillary bundles for cell cultures: No. 1. (U.S. Patent No. 4,184, 922), January 22, 1980. Knazek, R.A., Gullino, P.M., and Frankel, D.S. Dual circuit woven artificial capillary bundles for cell cultures: No. 2. (U.S. Patent No. 4,200, 689), April 29, 1980.

179. HORMONE PRODUCTION BY PITUITARY TUMOR CELLS

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Several clonal strains of rat pituitary tumor cells (GH-cells) synthesize and secrete into the medium different amounts of two hormones, prolactin (PRL) and growth hormone (GH). The synthesis of these two proteins can be further modulated by externally added physiological and pharmacological agents. Understanding of the mechanism/s involved in the graded basal level production of the hormone prolactin (PRL) and the regulation of its synthesis by thyrotropin releasing hormone (TRH), hydrocortisone (HC), estradiol (E2) and the drug 5-bromodeoxyuridine (BrdUrd) in GH-cells is the primary objective of this proposal.

Though the model cell system utilized in this investigation is not composed of normal cells, it still responds to physiological agents such as TRH, HC and E2 in the same manner as does the normal pituitary gland. Since distinct cell types are believed to produce specific hormones in the normal pituitary gland, the GH-subclones which produce two hormones (PRL and GH) provide a novel model system for the understanding of mechanisms whereby neoplastic cells can express a portion of the genetic information that is kept under strict control in normal cells. The synthesis of PRL by a distinct type of cells in the normal pituitary gland represents a differentiated function. The different GH-subclones which produce vastly different amounts of PRL, provide an ideal system for the investigation of the cellular mechanisms which lead to the regulated synthesis of such a cell specific protein and thus provide novel means to understand the process of cellular differentiation. The striking effect of BrdUrd on PRL synthesis will be utilized to obtain useful information on the nature of hormone receptor interactions with specific DNA binding sites. Cell-free systems in which events resembling specific RNA and protein synthesis occur, are established in this Laboratory. Such cell-free systems will be utilized to locate the control points of regulated synthesis of a hormone in pituitary tumor cells.

180. STEROID RECEPTORS IN MOUSE CELLS CULTURED IN VITRO

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Glucocorticoid receptors in intact cells can be inactivated to a state that does not bind steroid and then rapidly reactivated to a steroid binding state. The process of inactivation appears to involve a dephosphorylation and reactivation of a phosphorylation of either the receptor protein itself or an associated binding factor. Activation in cytosol preparations is stimulated by a heat stable factor that also stabilizes the unbound receptor to inactivation by incubation at 25 degrees. In the proposed work we will focus on the purification and characterization of the heat stable factor. A second focus is to demonstrate transfer of radiolabeled phosphate from ATP to a steroid-binding moiety.

BIBLIOGRAPHIC REFERENCES: Pratt, W.B., Sando, J.J., and Nielsen, C.J.: Glucocorticoid receptor inactivation and activation by phosphorylation mechanisms in steroid hormone receptor systems, ed. by W.W. Leavitt and J.H. Clark. New York:Plenum, 1979, pp. 343-356. Quasney, M.W., Bissen, S.T., Dahmer, M.K., Leach, K.L., and Pratt, W.B.: The use of molybdate permits improved resolution of untransformed glucocorticoid receptors from the transformed state. Abstracts of the 62nd Ann. Mtg. of the Endocrine Soc., 1980, in press.

4. UPTAKE AND TRANSPORT OF NUTRIENTS

181. PURINE TRANSPORT BY MEMBRANE VESICLES OF CANCER CELL LINES

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The vesicle transport system developed in previous years to assess the role of transport in cancer is extended to study the following areas: 1. The biochemical group translocation transport mechanisms described for inosine and hypoxanthine uptake will be further analyzed using double isotope and other analytic techniques not previously applied. 2. The classical non-group translocation transport mechanisms that appear to coexist in many cells will be studied along with the above enzyme-mediated pathways in order to elucidate the role of each mechanism. 3. Specific mutants with altered uptake and purine utilization previously isolated in this laboratory and elsewhere will be employed to facilitate the proposed studies. 4. Cell growth, membrane isolation, transport assay, enzyme purification and surface topology (by iodination and serology) will be the principal methods employed.

182. METHOTREXATE UPTAKE AND METABOLISM IN HEPATIC CELLS

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The proposed study is designed to elucidate the transport and metabolism of methotrexate and the folate coenzymes in hepatic tissue. A monolayer culture system of rat hepatocytes and a rat hepatoma (H35) cell line will be used to examine the transport processes. A comparative assessment will be made between the normal and transformed cells and the normal cells will be examined to determine the properties that allow these cells to act as the storage site for folates. A transport resistant line of the H35 cells has been developed and the properites of the cells will be compared with the normal H35 cells. Both hepatocytes and especially H35 cells catalyze a rapid conversion of methotrexate to polyglutamate derivatives. The properties of this reaction will be studied with cells in culture and cell free extracts to understand the enzymology and regulation of the reaction. We will also assess the contribution that the polyglutamates make to the chemotherapeutic action of methotrexate in H35 cells. Methotrexate bound to macromolecular carriers will be examined to determine their comparative effectiveness against normal and resistant H35 cells.

BIBLIOGRAPHIC REFERENCES: Galivan J.H. Evidence for the cytotoxic activity of polyglutamate derivatives of methotrexate. Mol. Pharmacol. 17, 105-110, 1980. Galivan, J., Tarentino, A., and Samsonoff, W. Membrane characteristics of primary hepatocyte cultures and a stable hepatocarcinoma cell line. Ann. N.Y. Acad. Sci. in press 1980.

183. REGULATION OF TRANSPORT IN CULTURED ANIMAL CELLS

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Oncogenic transformation of animal cell cultures and sugar deprivation result in markedly enhanced nutrient transport or uptake. How rates of transport are controlled is not known and how increased nutrient uptake relates to neoplastic potential has not been determined. In this proposal efforts to clarify these relationships focus on uptake regulation and utilize two different cell culture systems: 1) regulation of uptake by non-tumorigenic and tumorigenic cells by manipulation of sugar concentrations in culture media and 2) uptake and transport regulation in a highly neoplastic line which has low hexose uptake and high amino acid transport. In the initial approach, separation of the general process of nutrient uptake into nutrient transport and nutrient metabolism is outlined. Using whole cells, emphasis is

placed on the uptake of 2-deoxy-D-glucose and D-galactose as models for hexose uptake systems and on the transport of cycloleucine as a model amino acid transport system. Inhibitors of protein synthesis (cycloheximide and puromycin) and hexose transport (cytochalasin B) are considered for use in studies of how protein turnover might control uptake systems. Plans for the use of these inhibitors in promoting large differences in uptake rates are also outlined. Efforts to label specific transport molecules are likewise planned based on large fluctuations in transport rates promoted by selective inhibition and recovery from inhibition. Regulation of amino acid transport systems (using analogues such as alpha-amino isobutyric acid) will also be examined, since glucose starvation, as well as oncogenic transformation, seems to 'induce' derepression also in this system. Studies of transport of sugars by plasma membrane vesicles derived from transformed and untransformed cells are planned. A scheme for carrier purification (principally for the galactose transport molecule) and methods for the detection (assay) of the carrier are described. Carrier mediated transport and subsequent metabolism of nutrients are the focus of the study relating to the highly tumorigenic line of BHK cells. This section attempts to correlate the degree of anomalous nutrient uptake with in situ neoplastic potential. Thus the focus of the effort is to get a more clear characterization of how a readily measured carrier system is regulated. (Text Abridged.)

5. GENETIC ANALYSIS OF BIOCHEMICAL EVENTS

184. NEOPLASIA-PREDISPOSING GENES IN CELL CULTURE

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Genes associated with specific autosomal recessive syndromes may predispose the heterozygous carrier to cancer and leukemia. Because heterozygotes for these genes are relatively common in the general population, some genes identified in this way are likely to be among the important human neoplasia-predisposing genes.

Fanconi's anemia (FA) and ataxia-telangiectasia are two autosomal recessive syndromes associated with cancer and leukemia in heterozygotes and homozygotes. A systematic procedure has been developed for analyzing the defect determined by the FA gene, based on the observation that several abnormalities of FA fibroblast cultures are corrected by co-cultivation with normal cells. This procedure may be used to analyze the basic metabolic abnormality of several important neoplasia-predisposing genes.

185. INVESTIGATION OF THE GENETICS OF METABOLIC COOPERATION IN CULTURED MAMMALIAN CELLS

SubakSharpe, J. H., Sherratt, D. J., Buultjens, T. E., MacDonald, C. M., Hooper, M. L., University of Glasgow, Inst. of Genetics, *Church St., Glasgow G11 5JS, Scotland, United Kingdom*

OBJECTIVE: To use genetic analysis to study th mechanism of metabolic cooperation and its role in vivo.

APPROACH: Techniques are being developed for the isolation of mutants defective in metabolic cooperation from a mouse embryonal carcinoma tissue culture line in a defined number of selective steps and for subsequent analysis of their junctional permeability, ultrastructural and developmental capacity. The selection technique used for mutant isolation is based on the phenomenon known as the 'kiss of death', in which cells defective in the enzyme HGPRT (hypoxanthine guanine phosphoribosyl transferase), and therefore normally resistant to the toxic purine analogue 6-thioguanine, are killed by this analogue when grown in contact with wild-type cells as a result of metabolic cooperation.

PROGRESS: The cell line used as the parent in this selection, PC13TG8, shows efficient metabolic cooperation and forms differentiated tumors on subcutaneous inoculation into syngeneic mice (Devel. Biol., 55, 271(1977). Mutants have been isolated in a two-step selection procedure; they show a considerably reduced ability to transfer nucleotides labelled with either 3H-hypoxanthine, 3H-adenine or 3H-thymidine. Their future characterization is in progress.

186. GENETIC AND BIOCHEMICAL ANALYSIS OF CELL BEHAVIOR

Gottesman, M. M., Cabral, F., LeCam, A., Vlahakis, G., Singh, T., Roth, C., Abraham, I., Nicolas, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Gene Regulation Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

We are utilizing the Chinese hamster ovary (CHO) fibroblast grown in vitro to study the genetics and biochemistry of some aspects of the behavior of cultural cells. Our work has emphasized cell adhesion, morphology, response to cyclic AMP, the role of cell surface antigens, receptors and internalization of their ligands in cell behavior, and the mechanism of cell transformation by tumor viruses. We have isolated a variety of different mutants with abnormal adhesion, abnormal morphology, or abnormal response to cyclic AMP and some of these have been characterized as to their biochenical defects. We have developed a system for isolating cell mutants resistant to transformation by the product of the src gene of Rous sarcoma virus. We are establishing general procedures for the isolation of mutants lacking specific cell surface receptors, and mutants unable to internalize specific ligands. These mutants are analyzed genetically using the techniques of somatic cell hybridization and gene transfer, and biochemically by classical enzymology, affinity labelling techniques and two-dimensional electrophoresis.

BIBLIOGRAPHIC REFERENCES: Gottesman, M.M., LeCam, A., Bukowski, M., and Pastan, I. Isolation of multiple classes of mutants of CHO cells resistant to cyclic AMP. Somat. Cell Genetics 6: 45-61, 1980. Cabral, F., Willingham, M.C., and Gottesman, M.M. Ultrastructural localization to 10 nm filaments of an insoluble 58 K protein in cultured fibroblasts. J. Histochem. Cytochem., in press, 1980.

187. BIOCHEMICAL AND GENETIC STUDIES OF THE SURFACE MEMBRANES

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This is part of a broader project. A summary of this subproject is not available.

6. OTHER METABOLIC ACTIVITIES

188. REGULATION OF BENZO(A)PYRENE METABOLISM IN HUMAN CELLS - STUDIES WITH MONOCLONAL ANTIBODIES TO CYTOCHROME P-450'S

Park, S. S., Gelboin, H. V., Cha, S., Miller, H., Robinson, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Metabolic Control Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Benzo(a)pyrene (BP) is oxidized by mixed function oxidases (aryl hydrocarbon hydroxylase, AHH) as an initial step for the formation of an ultimate carcinogen, 7,8-diol-9,10-epoxide with the function of epoxide hydratase (EH). Human monocytes, lymphocytes and human lung cell line, A549, were grown in the presence of benzo(a)anthracene (BA) as an inducer. Microsomes were prepared from human liver and placenta of heavily smoking women. When the cells were tested for AHH and EH activity, the levels of enzymes were 2-4 times those of the uninduced cell activities. The human placenta microsomes also retained high AHH activity. However, the AHH activity of human placenta microsomes was reduced by 21%, of monocytes induced by BA by 5.4% and of rabbit microsomes induced by phenobarbital (PB) by 27% at 1:2 dilutions by preincubating with monoclonal antibodies to liver microsomal cytochrome P-450 LM2 of rabbits induced by PB. The purpose of this work is to investigate the appearance of a specific predominant form of isozymes involved upon induction using monoclonal antibodies as tools.

189. THE INFLUENCE OF AGE ON PHOSPHATIDYLINOSITOL TURNOVER

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OBJECTIVE: The objective of this project is to study the turnover of phospholipids in proliferating keratinocytes and fibroblasts in culture.

APPROACH: Keratinocytes cultured on lethally irradiated 3T3 fibroblasts will be exposed to 12-0-tetradecanoylphorbol-13-acetate (TPA) and to epidermal growth factor (EGF). Fibroblasts in culture will be exposed to TPA and to EGF. Alterations in cell phospholipids will be studied with the use of radioisotopes and thin layer chromatography.

190. METABOLISM OF NEOPLASTIC CELLS

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Effects of antimetabolites on neoplastic cell growth in vitro continue to be investigated, with the aim of developing a reliable in vitro 'therapeutic trial' for antitumor agents.

191. STUDY OF MECHANISMS INVOLVED IN PURINE ANALOGUE RESISTANCE IN CHINESE HAMSTER CELLS

Fox, M., Paterson Lab., Dept. of Experimental Chemotherapy, *550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom*

OBJECTIVE: To determine whether resistance to purine analogues arises by genetic or epigenetic mechanisms or both.

APPROACH: Purine analogue resistant lines isolated from different wild-type V70 lines after either single or multi-step selection procedures will be phenotypically characterized by their subsequent ability, or otherwise, to grow in HAT medium and high concentrations of azaguanine and thioguanine. Patterns of cross-resistance will be thus established and highly resistant lines will be characterized further with respect to the kinetic properties of HGPRT, i.e., affinity for PRPP, normal purine bases and purine analogues. Resistant lines will be further characterized by determination of ionic mobility of HGPRT in polyacrylamide gels, presence or absence of CRM, revertibility and heat sensitivity of the enzyme. In this way a distinction will be made between lines which possess or do not possess altered gene products. Amongst those lines which possess altered gene products, i.e., carry mutations in their structural genes, the nature of the alteration will be further elucidated by reversion studies and further biochemical analysis of the mutant enzyme.

PROGRESS: Two classes of purine analogue resistant lines have now been identified. Lines isolated in a single step after exposure to high concentrations of purine analogues invariably have detectably altered gene products as determined kinetically or by polyacrylamide gel electrophoresis. Those isolated after chronic exposure show reduced HGPRT levels, but the kinetic behavior and ionic mobility of the enzyme is identical with that of the wild-type enzyme. Lines of this latter class may be the result of epigenetic modulation of HGPRT activity while those of the former carry mutations in their structural genes. Study of the former class of lines is continuing.

192. PURIFICATION AND ANALYSIS OF ADIPOGENIC FACTOR INTERACTIONS WITH 3T3 CELLS

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The major aim of the proposed study is to lay the groundwork necessary for the elucidation of the mechanism of the adipose conversion of the 3T3 preadipocyte-fibroblast. To do this, the factor required for the conversion will be purified to homogeneity and radiolabelled. This will enable determination of (1) receptor number and dissociation constant, (2) the Ka and concentration of factor internalized, and (3) the Km for adipose conversion. Analysis of these parameters in growing and resting preadipocytes and in mature adipocytes should yield information on how cells are programmed to respond to differentiation. The process of factor internalization will be examined, to determine if it is necessary for degradation and/or factor action. Internalization will be blocked by methylamine or cellular mutation and conversion measured. Adipose conversion in the absence of factor internalization will show that internalization is only a method of degradation.

Once this system is well characterized it should be possible to develop an assay for the event causing conversion and to isolate that molecule involved. If for example changes in the phosphorylation of nonhistone proteins occur, the kinase or phosphatase involved would be isolated and its control studied.

193. DIFFERENTIATION OF IN VITRO CULTURES FROM AMPHIBIAN MESODERM

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Every cell of the amphibian embryo contains its own yolk granules and it is for this reason that explants continue to undergo cell division and differentiation for several days in simple buffered salt solutions in the absence of added nutrients. However, mesodermal explants differentiate much better when they are cultured inside ectodermal jackets than they will on their own. This has two disadvantages: 1. although the bulk medium is defined and simple the medium in immediate contact with the mesodermal cells is not defined; 2. it is not possible to observe the cells continuously during the culture period. We have therefore been looking for an alternative culture method for this type of experiment.

Differentiation does not seem to occur in single cell preparations or in monolayers. However, we have achieved some success by using a new salt solution of higher tonicity than the previous ones and allowing the cells to spread out from explants onto tissue culture plastic. The addition of nutrient media or serum is not necessary. Under these conditions differentiation occurs in about 70 percent of cultures and three distinct cell types can be distinguished. One has been identified as striated muscle by transmission electron microscopy and the other two are probably notochord and blood cells. We intend to investigate the effects of different substrata and matrices for the cells to see whether any further improvement is possible.

E. CULTURED CELLS AS A TEST SYSTEM FOR CHEMICAL CARCINOGENESIS

194. AN EVALUATION OF THE SISTER CHROMATID EXCHANGE METHOD IN MAMMALIAN (INCLUDING HUMAN) CELLS AS A SCREENING SYSTEM FOR CARCINOGENS

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OBJECTIVE: To evaluate the sister chromatid exchange test for identification of chemical carcinogens or mutagens.

APPROACH: To investigate as many chemicals as is feasible using the SCE test and to compare this information with the known carcinogenicity/mutagenicity of these compounds. To optimize the conditions of the test, such as chemical exposure time, preparation and use of S9-Mix, and use of cells with inherent metabolic ability so as to achieve maximum sensitivity.

PROGRESS: A large number of chemicals have been studied using this technique. The effects of duration of exposure to chemical or SCE frequency in cultured cells have been established. Experiments are continuing to establish optimum conditions for preparation and use of S9-Mix.

195. CARCINOGENESIS SCREENING USING CELL CULTURE ASSAYS

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OBJECTIVE: To evaluate the utility and reliability of in vitro cell transformation systems as initial estimation in carcinogenic potential of some compounds from occupational field. The assays will be performed without and with in vitro or in vivo metabolic activation. In vitro transformation will be measured by morphological changes, ability to grow in soft agar and transplantation to appropriated hosts.

196. THE BIOLOGICAL EFFECTS OF CARCINOGENS ON CULTURED HUMAN EPIDERMAL KERATINOCYTES

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OBJECTIVE: To investigate the role of benzo(a)pyrene and tumor promoters in the neoplastic transformation of cultured human epidermal cells.

APPROACH: Epidermal cells cultivated using fibroblast support or m[illegible]n containing small amounts of calcium will be used to investigate the following: 1) The action of tumor promoters on epidermal multiplication, differentiation and metabolic cooperation. 2) The properties of basal and squamous cell carcinoma cells, with particular reference to their ability to form established lines, and to give rise to differentiated tumors when inoculated into immunosuppressed mice. 3) The ability of BP and BP 7,8 dihydrodiol 9,10 oxides to reproducibly transform diploid human epidermal cells into neoplastic cells and the role of tumor promoters in this transformation. 4) The role of chromosome rearrangements in human epidermal carcinogenesis.

PROGRESS: BP is metabolized by epidermal cells cultured using fibroblast support, and the BP 7,8 dihydrodiol 9,10 oxide is the major DNA-reaching metabolite (Parkinson and Newbold, 1980, Int. J. Cancer, 26, 289-299).

197. CHEMICAL CARCINOGEN-INDUCED DNA REPAIR IN HUMAN CELLS

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The overall objective of this project is to understand more about how human cells repair DNA damage induced by carcinogens. During the past year we have focused on two major sets of specific aims. The first involves additional studies on the role of chromatin structure in repair, while the second relates to identifying individual enzymes and other proteins which are involved in DNA excision repair in eukaryotic cells. Work on the role of chromatin structure in excision repair has centered around the phenomenon of rearrangement, i.e., the finding that initially nucleotides incorporated during repair synthesis are staphylococcal nuclease sensitive, and during a subsequent chase period, they become nuclease resistant (appear in nucleosome core particles). With some agents such as 7-bromomethylbenzanthracene and ultraviolet radiation, damage is relatively uniformly distributed between core DNA and linker DNA; with 7-BMBA, rates of removal are similar in core and linker regions. Two possible models might explain these data: one is an unfolding-refolding model of rearrangement in which there are changes in chromatin conformation during DNA excision repair; these changes render the regions undergoing repair temporarily nuclease sensitive, and subsequently they become resistant. A second model suggests that the repair-incorporated nucleotides become nuclease resistant because of sliding. We have tried several approaches to isolate and purify factors involved in repair. One set of experiments involves utilizing a permeable cell system developed by us (Roberts and Lieberman, Biochemistry 18:4499, 1979) to complement permeable xeroderma pigmentosum cells. We have recently isolated a carcinogen-DNA binding protein: the protein recognizes and binds to DNA modified by N-acetoxy-2-acetyl-aminofluorene (NA-AAF), methylnitrosourea, and methylmethanesulfonate, but shows little binding to normal DNA (Moranelli and Lieberman, PNAS 77:3201, 1980). At present the function of this protein is unknown, but we are actively working towards its further purification and characterization.

BIBLIOGRAPHIC REFERENCES: Smerdon, M.J., and Lieberman, M.W. Comparison of the distributions within chromatin of DNA repair synthesis occurring at different times after UV radiation. Biochemistry 19:2992-3000, 1980. Moranelli, F., and Lieberman, M.W. Recognition of chemical carcinogen-modified DNA by a DNA-binding protein. Proc. Natl. Acad. Sci. USA 77: 3201-3205, 1980.

198. MECHANISMS OF CARCINOGENESIS IN CELL CULTURE

Bertram, J. S., Libby, P. R., McCormick, P. J., Roswell Park Memorial Inst., Dept. of Experimental Therapeutics, *666 Elm St., Buffalo, New York,* 14263, United States of America

We propose to study the mechanisms whereby actinomycin D and caffeine produce modulations in the transformation frequency in vitro of cultured C3H10T 1/2 cells induced by polycyclic aromatic hydrocarbons. We will study the effects of these dose modifiers on a) cell cycle progression, by measuring rates of DNA and RNA synthesis and by flow microfluorometry, b) metabolism of benzpyrene using high pressure liquid chromatography, c) binding of benzpyrene to DNA using enzymic digestion and column chromatography. We will also attempt to explain the time dependency for action of these dose modifiers by studying the stability of carcinogen induced DNA lesions and the rate of production of DNA strand breaks by a sensitive new method utilizing alkaline sucrose sedimentation analysis. We finally propose to clarify the effects of cell cycle progression and further clarify factors affecting malignant transformation by studying the cell cycle phase specificity for malignant transformation of pro- and ultimate carcinogens inducing both x-ray and UV type damage. For these studies we will develop techniques for the harvesting of mitotic 10T 1/2 cells.

BIBLIOGRAPHIC REFERENCES: Bertram, J.S., Libby, P.R., and Merriman, R.L. Modulations of carcinogen-induced transformation by Actinomycin D in the C3H/10T 1/2 cell line. J. NCI, 64, 1393, 1980.

199. IN VITRO CHEMICAL CARCINOGENESIS

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Hybrids formed by polyethylene glycol fusion of rat liver cells containing abnormal cytoplasmic aggregates of intermediate filaments with murine hepatoma cells which show a normal distribution of intermediate filaments will be examined in an effort to determine the type and extent of genetic control involved in maintaining the altered cytoskeletal structure. The hybrids will, furthermore, be analyzed for their ability to synthesize albumin, alpha fetoprotein and gammaglutamyltranspeptidase.

We will conduct experiments in which freshly isolated rat liver cells, as well as an established normal rat liver cell line, will be incubated with diethylnitrosamine and N-methyl-N'-nitro-N-nitrosoguanine followed by the promoter 12-0-tetradecanoyl-phorbol-13 acetate. The progeny will be examined for mutagenic changes and for neoplastic transformation.

BIBLIOGRAPHIC REFERENCES: Borenfreund, E., Higgins, P.J. and Peterson, C. Intermediate size filaments from cultured rat liver tumor cells with Mallory body-like cytoplasmic abnormalities. J. Natl. Cancer Inst. 64:323-333, 1980. Borenfreund, E., Schmid, E., Bendich, A., Franke, W.W. Constitutive aggregates of intermediate-sized filaments of the vimentin and the cytokeratin type in cultured hepatoma cells and their dispersal by butyrate. Exper. Cell Res., 1980, in press.

200. AN INVESTIGATION OF THE CARCINOGENIC ACTION OF NITROSAMIDES AND RELATED COMPOUNDS USING TISSUE CULTURE TECHNIQUES

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OBJECTIVE: To identify changes in cell properties which occur during nitrosamide-induced carcinogenesis in the rat brain and to investigate the relationship of these to malignant transformation.

APPROACH: Pregnant rats (inbred BD IX line) are injected with ethylnitrosourea (ENU) on the 15th or 16th day of pregnancy. This transplacental exposure results in the induction of cerebral gliomas in a large proportion of the offspring. Cultures are prepared from the brains of foetuses or cerebra of offspring at a number of times after exposure to ENU but before the appearance of a visible tumor. Control cultures are prepared from animals exposed transplacentally to buffer. The cultures are examined for several features found characteristic of cells cultured from gross gliomas, e.g., tumorigenicity in newborn syngeneic animals, specific cellular morphology (phase contrast microscopy), growth in agar, fibrinolytic activity, altered surface morphology (scanning electron microscopy). Particular attention is being paid to the possibility that these characteristics are acquired at different times following exposure to ENU.

PROGRESS: The sequential in vivo-in vitro approach has shown that the presence of malignant cells can readily be demonstrated about half-way through the latent period. However, potentially malignant cells which only become tumorigenic after many months in culture are already present two days after exposure to ENU. These results are consistent with a multistage

process of carcinogenesis. Further results suggest that fibrinolytic activity precedes the ability to form colonies in agar. A more detailed investigation of the sequence of changes and their relationship to each other is now being undertaken.

201. MODULATION OF AHH AND EH IN ANIMAL TISSUES AND IN CELL CULTURE BY CIGARETTE SMOKE CONDENSATE (ABBREV)

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In many instances, the selective toxic effect of carcinogens on certain organs can be attributed to the activities of different drug metabolizing enzymes present in those tissues. In particular, this effect could be due to the balance existing between the activating enzymes responsible for the synthesis of reactive intermediates and the inactivating systems producing non-toxic metabolites. Moreover, the sensitivity of a given tissue or species to chemical carcinogens not only depends on the basal activity of those different enzymes but also on their genetic susceptibility to be induced by various chemicals.

Both in animals and tissue culture, experimental conditions exist which selectively induce the aryl hydrocarbon hydroxylase (AHH) and epoxide hydratase (EH) activities, these being two key enzymes in the metabolism of polycyclic aromatic hydrocarbons. For our experimentation we used rats, different strains of mice, primary fetal rat liver cell culture and different hepatoma cell lines as models. In addition, chemicals such as cigarette smoke condensate, trans-stilbene oxide, antioxydants, polycyclic hydrocarbons and other monooxygenase inducers capable of modifying the studied enzymes were implemented.

Our specific aims are: 1. To understand the reasons for the selective action of inducers (CSC, TSO, PB, BP, etc.). 2. To determine the precise quantitative and qualitative role of the different enzymes (namely AHH, EH, GSTr) involved in polycyclic hydrocarbon metabolism in an integrated system (i.e., cell culture). 3. To investigate the biochemical mechanisms of AHH and EH regulation at a molecular level.

Our long-term objective includes the extrapolation of our data to the human situation. As a first approach to this problem, we are presently studying the biochemical behavior of the main drug metabolizing enzymes in animal skin as well as in keratinocytes and skin fibroblasts in culture. Later, in collaboration with Professor Prunieras (Paris), we will extend this work to include humans by utilizing of human epidermal cells in culture.

202. INTESTINAL CELLS - GROWTH REGULATION AND CARCINOGENESIS

Quaroni, A., Isselbacher, K. J., May, R. J., Hauri, H., Harvard University, Boston Campus, Massachusetts General Hospital, Medical Services, *Fruit St., Boston, Massachusetts*, 02114, United States of America

The overall objectives of the proposed research are: (1) to study the growth regulation of normal versus tumor intestinal cells in vitro. These studies will focus on the effects of various hormones (hydrocortisone, thyroxin, glucagon, insulin, gastrin, etc.) and of an apparently specific growth inhibitor partially purified from intestinal villous cells on normal intestinal epithelial cells and on tumor cell lines derived from dimethylhydrazine-induced tumors of the small and large intestine. (2) To study the susceptibility to chemical carcinogenesis of normal epithelial cells derived from different portions of the rat intestine. Malignant transformation will be induced by addition of various chemicals (benzo(a)pyrene, 1, 2-dimethylbenz (a) anthracene, methylnitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine) and monitored by following many possible cellular changes (a different morphology, increased saturation density, growth in soft agar, plasminogen activator production, decreased fibronectin deposition). (3) To attempt to establish normal epithelial cell lines from human small intestine; if obtained, these cell lines will be characterized by morphologic and immunologic criteria similar to those used in our studies of rat intestinal cells.

BIBLIOGRAPHIC REFERENCES: Quaroni, A., Wands, J., Trelstad, R.L., and Isselbacher, K.J., Epithelial cell cultures from rat small intestine. Characterization by morphologic and immunologic criteria. J. Cell Biol. 80:248, 1979. Quaroni, A., Kirsch, K. and Weiser, M.M. Synthesis of Membrane glycoproteins in rat small intestinal villus cells. Redistribution of in vivo (1,5,6-3H)-L-fucose labeled membrane glycoproteins among Golgi, lateral-basal and microvillus membranes. Biochem. J. 182:203, 1979.

203. ROLE OF MUTATOR GENES IN CHEMICAL CARCINOGENESIS

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OBJECTIVE: Develop a methodology to isolate and characterize DNA repair mutant lines and to use these mutants for carcinogen screening.

APPROACH: Control and carinogen treated populations of Lew A(1) cats will be examined for the appearance of transformed cells. Populations known to contain preneoplastic and neoplastic cells will be identified. These cell lines will then be used to test various carcinogens such as ozide.

F. GROWTH STUDIES ON CELL CULTURES

204. CYSTATHIONASE EXPRESSION IN HUMAN NEOPLASIA

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It has been shown that a number of human and rodent leukemic cell lines are dependent on preformed cysteine in tissue culture media and that they remain growth arrested in the presence of cystathionine, the precusor of cysteine. Substantial evidence exists to suggest that the cysteine auxotrophy of such cells (cys-negative) results in part from a depletion of cystathionase, the enzyme responsible for the conversion of cystathionine to cysteine. Human lymphoblastoid cell lines derived from nonleukemic individuals maintain normal growth in the absence of cysteine, provided cystathionine is present and they have easily detectable cystathionase (cys-positive). I have recently shown that cysteine auxotrophy may be expressed in vivo by leukemic cells. I have developed a murine hybridoma cell line which secretes a monospecific antibody to human cystathionase. A major objective of the proposed research will be to use my reagent to quantify the content of cystathionase in fresh human leukemic, lymphoma, and solid tumor cells and compare the levels to those of the relevant normal tissues. The techniques used will include immunofluorescence and immunoperoxidase staining of relevant cells as well as rocket immunoelectrophoresis and radioimmunoassay. A second objective will be to detemine the sensitivity of the earliest committed stem cells from human bone marrow (CFU-C) to cysteine depletion in the presence and absence of cystathionine. The sensitivity of such cells will be compared to their cystathionase content, and the expression of cystathionase in the various stages of the maturing normal granulocyte and gut epithelial cell will be delineated. Finally, we propose to explore the relative toxicity of various cysteine analogues to both cys-positive and cys-negative cells. The potential for selective cystathionine 'rescue' of cys-positive cells in the presence of toxic cystein analogues will be examined by in vitro tissue culture techniques.

205. INTESTINAL CELLS - GROWTH REGULATION AND CARCINOGENESIS

Quaroni, A., Isselbacher, K. J., May, R. J., Hauri, H., Harvard University, Boston Campus, Massachusetts General Hospital, Medical Services, *Fruit St., Boston, Massachusetts*, 02114, United States of America

The summary for this project is under entry no. 202.

206. GROWTH CONTROL OF NORMAL AND MALIGNANT FIBROBLASTS

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OBJECTIVE: To study the role of the protein synthetic machinery in the control of normal and malignant cell proliferation.

APPROACH: In order to test the hypothesis that regulation of the level of the gross protein synthetic machinery of mammalian cells could regulate cellular proliferation, a number of cell mutants conditionally deficient in the function of the protein synthetic machinery have been isolated. These are the

aminoacyl-tRNa synthetase mutants for a number of different amino acids, and mutants in the biosynthesis of asparagine, all in the CHO cell system. To examine the effect of expression of aminoacyl-tRNA synthetase mutations on both normal and transformed cell backgrounds, a line with almost normal growth characteristics was isolated from an exceptionally non-leaky ts leucyl-tRNA synthetase mutant of transformed CHO cells. The effect of rapid generation of uncharged tRNA can now be studied in both transformed and quasi-normal cells.

These mutants have also been used to develop a rapid, sensitive assay for errors in protein synthesis using two-dimensional size electrofocusing gels. The probability of mistranslation during amino acid starvation of both normal and malignant cells is now under investigation.

207. REGULATION OF THE MAMMALIAN CELL CYCLE

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The current concept of cell regulation has led to a hypothesis that is difficult to analyze biochemically and biologically. I have developed a culture system which allows experimental study of growth cycle regulation, and I have shown the serious limitations of the previously used cell systems for the study of growth control.

Serum control of cellular growth has been shown to be regulated in two discrete phases: competence and progression. I have proposed the study of the processes involved in competence and progression. The understanding of these processes will provide valuable knowledge concerning cell regulation.

Competence is induced by a platelet derived mitogen. The biochemical processes by which competence is induced are not known. The experiments proposed in this grant are designed to elucidate the processes by which competence is formed. Since SV40 abrogates the cellular growth requirement for the platelet mitogen, it is important to determine if transformation induces the normal biochemical process which renders the cell competent.

This proposed work will allow the study, cellular and biochemical, of the process of commitment (that point at which the cell is committed to DNA synthesis). The analysis of this process will greatly enhance our knowledge of cell cycle control.

I have proposed work to show how viral transformation alters normal cell regulation. These findings can lead to a model that describes the mechanisms by which normal cell regulation is lost.

The understanding of how the growth cycle is regulated and which areas of regulation can be controlled may provide the means to control or arrest cells that have lost their normal control.

Not only does this research grant have significance in regulation and growth control research, but it also will provide needed information on a hormonal response mechanism. The platelet derived mitogen can be classified as a hormone. Knowledge of the mechanism by which this factor induces RNA synthesis and competence can provide the understanding of the biochemical mechanism by which a hormone 'triggers' or regulates a biological response.

208. FACTORS REQUIRED FOR MAMMALIAN CELL DIVISION

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The long-range objective is to understand enough about the factors that control mammalian cell growth to be able to apply this knowledge to the control of malignant growth. As a result of recent work, a broad picture is now available of the control of growth of mammalian cells. Application of this knowledge requires additional information and experimentation. Detailed information is needed on the specific factors that control the growth of the epithelial cells that are most important in the origin of tumors, in order to design rational procedures to restrict tumor growth. It is important to know whether these epithelial cells produce inhibitors of their own growth, such as the growth inhibitors that are produced by kidney epithelial cells. Isolation of the kidney determines whether the inhibitors can be used to restrict the growth of kidney tumors. These questions will be studied using methods of cell culture to investigate growth controls, using ultrafiltration, chromatography and other biochemical procedures in the isolation of the inhibitors, and using cell free systems, cell culture and work in intact animals to investigate the properties of the inhibitors.

BIBLIOGRAPHIC REFERENCES: Robert W. Holley, Control of growth of kidney epithelial cells. In: Control Mechanisms in Animal Cells, edited by L. Jimenez de Asua et al., Raven Press, New York, pp. 15-25, 1980. Robert W. Holley, P. Bohlen, R. Fava, J. H. Bladwin, G. Kleeman and R. Armour. Purification of kidney epithelial cell growth inhibitors. Proc. Natl. Acad. Sci USA 77, 5989-5992 (1980).

209. INVESTIGATION OF THE MECHANISM OF ADHESION BETWEEN MAMMALIAN CELLS GROWN IN CULTURE

Edwards, J. G., Dysart, J. M., University of Glasgow, Dept. of Cell Biology, *Glasgow G12 8QQ, Scotland, United Kingdom*

OBJECTIVE: To identify the molecular species controlling the adhesiveness of certain cultured mammalian cells, particularly BHK21 cells, and to find an explanation in molecular terms for the reduction of this adhesiveness which follows transformation of the cells by tumour viruses.

APPROACH: Adhesiveness of fibroblastic cells is measured in a variety of assays: aggregation, short and long term, in suspension; attachment to cell sheets and protein coated substrates. The effects on adhesiveness of agents which modify the cell surface, particularly enzymes such as trypsin and neuraminidase, or which affect adhesiveness via intracellular activities are being investigated. A series of cell variants with altered adhesive properties has been assembled and proteins of their surface membrane analysed.

PROGRESS: 1. Short-term aggregation of BHK21 cells is tubulin-dependent. (Nature 248: 239, 1974) 2. Unlike attachment to non-cellular substrates it is largely independent of divalent cations in the medium (although it is stimulated by Mn ion) and cellular energy metabolism. Aggregation depends on trypsin-sensitive protein outside the cells (J. Cell Sci. 19: 653, 1975). 3. Cell variants selected for resistance to the toxic lectin ricin show reduced adhesiveness, both cell to cell and cell to substrate, implicating surface glycosylation in the phenomena. (Nature 264: 66, 1976). 4. All transformed cells show reduced aggregation in a long-term assay, but only some of these are reduced in short-term adhesiveness.

210. ROLE OF IRON IN THE CONTROL OF NORMAL AND MALIGNANT CELL GROWTH

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One of the major problems confronting the molecular biologist is the understanding of the molecular events that control growth of mammalian cells. In general, the multiplication of cultured untransformed mammalian cells requires serum and in defined media (media without serum) it requires the presence of the serum iron carrier glycoprotein transferrin (Tr). Many transformed cells have lower serum requirements than the untransformed counterparts and some which proliferate in defined media do not require Tr. From these considerations, it can be suggested that some transformed cells must be able to regulate iron transport by a mechanism different from Tr mediated iron uptake. Thus, the understanding of differences and similarities in the mechanisms of iron transport between normal and transformed cells may be of great significance for the understanding of transformation. In order to study this problem two major complementary lines of work are in progress: 1) the elucidation of the interaction of Tr with specific receptor, particularly in normal cells, and its relation to cell proliferation; 2) identification of ways in which transformed cells transport iron in the absence of Tr.

211. TAXOL (NCS 125,973) PRODUCTION BY CELL CULTURE

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During the next year the following aspects of the study will be pursued: The growth rate of Taxus cultures will be improved by modification of the medium composition and environmental conditions. Cultures will be established from growing plants rather than from embryos. The cytotoxic material in cell cultures will be measured and those with the highest yield will be selected for further study.

212. THE RELATIONSHIPS BETWEEN ANCHORAGE DEPENDENCE AND SURFACE AREA IN HAMSTER AND MOUSE FIBROBLASTS

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It has long been known that the growth of cultured cells is determined by factors in the medium, but this does not explain all the special features of their growth, such as their dependence on attachment to a solid surface. One hypothesis is that the interaction between a cell and its medium is limited by the area of its surface.

We have now shown that area can account for the differences between attached and suspended growth in four different kinds of fibroblast. In those cases where the spread area can be adjusted to equal that of suspended cells, growth is unaffected by attachment and in all cases growth ceases in suspended colonies when their surface-to-volume ratio becomes equal to that of attached monolayers. This is true not only of some maintained cell lines but also of an unselected primary cell population. It therefore seems likely that surface area may be an important general mechanism modulating the rate at which fibroblasts collect growth promoters from the medium.

We are therefore now trying to determine how far area can explain the regulation of growth of cultured cells in general. Since attachment can be arranged to cause intercellular contact without changes in area, it should then be possible to identify unequivocally any cases where such contact has an effect on proliferation, and to determine how these two mechanisms are altered in transformation by carcinogens.

213. A COMPARISON OF THE HORMONAL REQUIREMENTS OF NORMAL AND NEOPLASTIC LEYDIG CELLS IN MONOLAYER CULTURE

Cooke, B. A., MageeBrown, R., University of London, Royal Free Hospital School of Medicine, Dept. of Biochemistry, *8 Hunter St., London WC1N 1BP, England, United Kingdom*

OBJECTIVE: To develop a primary culture system for investigating the regulation of functional activity and growth of normal testicular and neoplastic Leydig cells.

APPROACH: Using purified Leydig cells from rat testes and from Leydig cell tumors the following will be investigated: 1) The hormones and factors necessary to maintain the LH-responsiveness of normal and neoplastic Leydig cells in primary monolayer culture. 2) The hormones and factors which control the growth of normal and neoplastic Leydig cells in culture.

214. GROWTH, METASTASES AND THE CELL SURFACE

Burger, M. M., Wiedmann, T. W., Ballmer, K., Universitat Basel, Dept. of Biochemistry, Biocenter, *Klingelbergstrasse 70, CH 4056 Basel, Switzerland*

OBJECTIVES: Search for relevance of cell surface to growth in cultures and to invasive growth and metastasis in vivo.

APPROACH: 1. Growth in vitro: Addition of a non-toxic derivative of the carbohydrate binding lectin concanavalin A to growing SV40-transformed mouse 3T3 fibroblasts inhibited their growth at lower concentration than was required to inhibit growth of the untransformed cells. The mechanisms of action of this cell surface modulation upon growth control will be investigated by the following approaches: Determination of the point in the cell cycle when the cells are afflicted, determination of whether cell migration is also influenced, whether cell-cell adhesion is influenced, whether the binding of growth factors from the serum is prevented from taking place, etc. 2. Growth and metastasis in vivo: Variants of melanoma cells (Fiedler's F-1) will be isolated. The variants should have an altered cell surface membrane (lectin-resistant variants or mutants) and will be tested for altered metastasis after i.p. and i.m. application. After establishing the biochemical surface alteration, biological parameters which might be relevant for metastasis and altered growth behavior in vivo (adhesion, migration, protease release, deformability, escape from the peritoneum, etc.) will be studied in vitro and in vivo.

PROGRESS: 3T3 cells are arrested by succinyl Con A in G1 while SV40 cells are shifting their cell cycle pattern towards more cells being in G1. Succinyl-Con A changes the anchorage independent transformed cells reversibly to anchorage dependent cells. The mechanism is still unclear (Science 201, 824, 1978).

A wheat germ agglutinin (WGA) resistant melanoma cell line was shown to metastasize less if administered i.p. (Nature 270, 437, 1979). The surface defect could be pinpointed as a decrease in WGA surface receptors, specifically the loss of two out of 4 sialic acids in a mannose containing tetra-antennary oligosaccharide which acquired two additional fucoses.

G. OTHER SPECIFIC BIOLOGIC APPLICATIONS

215. CULTURE OF NORMAL AND MALIGNANT CELLS IN CONDITIONS SIMULATING IN VIVO

Rose, S., Research Bio-Response, *2200 Webster St., San Francisco, California,* 94115, United States of America

OBJECTIVE: To create an environment which will allow a variety of cells to be cultured in conditions simulating in vivo.

APPROACH: The cells are cultured in fresh flowing cell free lymph which has been generated at an induced increased capillant filtration.

PROGRESS: The lens of the ETR has been cultured and has been maintained in a transparent state for several weeks. In contrast the lens becomes opaque in 12-24 hours in standard culture.

216. DETECTION OF MYCOPLASMA IN CELL CULTURES

Hales, A. C., House, W., Imperial Cancer Research Fund, Central Cell Services, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

Routine methods used for the detection of mycoplasma in cell cultures are: aceto-orcein staining; growth on agar and cell-enriched agar. These methods have proved to be quicker and more sensitive than use of the scanning electron microscope.

We are conducting tests on agar cultures using the 'Gaspak' incubation system to compare results with routine incubation in an atmosphere of 5 percent CO2 in nitrogen.

217. UPGRADING OF A FLOW FLUORIMETER CELL SORTER WITH A SINGLE CELL DEPOSITION SYSTEM

Bolund, L. A., Aarhus Universitet, Inst. of Human Genetics, *DK8000 Aarhus C, Denmark*

We intend to upgrade an instrument for fluorescence activated cell sorting with a system for sorting single cells. Cells that pass one by one through a laser beam are analyzed with respect to fluorescence and light scatter and are then included in microdrops. The new equipment makes it possible, when a cell with required properties is passing, to charge the microdroplet with the cell so that this and only this cell with the aid of an electrical field can be made to deviate to a separate culture well where by proliferation, it can give rise to a homogenous cell population (clone).

Several research groups in Aarhus have started to make hybridomas that produce monospecific antibodies against different types of normal as well as virus and tumor cell specific molecular structures. Such antibodies tend to become invaluable tools in basic research and clinical work.

The hybridomas are produced by fusion of milt lymphocytes from an immunized animal with tissue cultured myeloma cells. A major problem with the technique is that only a small proportion of the hybrid cells obtained produce the relevant antibody. The selection and cloning of the antibody-producing cells thus required can be achieved much more simply and efficiently than previously with the equipment granted.

218. ARTIFICIAL CAPILLARY CELL CULTURE

Wieman, M., Roger Williams General Hospital, *825 Chalkstone Ave., Providence, Rhode Island,* 02908, United States of America

This is part of a broader project. A summary of this subproject is not available.

219. DETECTION AND IDENTIFICATION OF TISSUE CULTURE CONTAMINANTS

Fogh, J. E., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

IV. BROAD STUDIES ON IN VITRO TECHNIQUES

220. IDENTIFICATION OF CELL CULTURES

Peterson, W. D., Child Research Center of Michigan, *3901 Beaubien Blvd., Detroit, Michigan*, 48201, United States of America

The use of tissue culture system in setting up model systems has been and will continue to be an important aspect of the Viral Oncology Program to study the possible role of viruses in the etiology of human cancer. The purpose of this project is to provide for maintenance of a facility which rapidly and successfully responds to the needs of investigators for characterization and identification of many diverse cell lines. This is to establish purity and species of origin. These tasks will require biochemical (isozyme), immunological (including immunofluorescent), and chromosomal analysis in various combinations.

221. DEVELOPMENT AND CHARACTERIZATION OF CELL SUBSTRATES FOR UTILIZATION IN CANCER RESEARCH AND ALLIED STUDIES

Vedros, N., Nelson Rees, W., U.S. Dept. of Defense, Navy, United States of America

OBJECTIVES: The Cell Culture Laboratory (CCL) is physically located at the Naval Biosciences Laboratory (NBL), Oakland, California. This project includes the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karyotyping of cell cultures, and performing biophysical, virological, and cytogenetic applied research.

MAJOR FINDINGS: During the reporting period, the contractor continued to initiate, grow, preserve, characterize, and distribute a variety of human and animal cells for utilization in cancer research. Procedures utilized are such that antibiotic-free cultivation is achieved in the majority of cases. Routine laboratory procedures are such that mycoplasma contamination occurs only at a very low frequency among the numerous cultures initiated, grown, and preserved in this laboratory.

During this reporting period, approximately 1,000 cell culture seed stocks (ampoules and flasks) were distributed to 100 individual scientists.

A number of valuable cell lines of tumor origin have been received from other laboratories and processed at CCL for distribution; among these were four of human origin. Approximately 20 laboratories have submitted 100 individual cell cultures for analysis of species and cell line purity. Approximately 33 percent were as purported and others involved intra- and inter-species contamination. Activities of this nature are projected to continue at the same or increased levels for the duration of the contract.

Morphologically abnormal cell lines are being compared to morphologically normal cells in terms of growth pattern, cell doubling time, saturation density, clonal growth on various substrates, karyology, and tumorigenicity in nude mice. Clonal growth studies are in progress on a variety of low passage, non-HeLa, human cancer cell lines in an effort to define the optimum nutrition and environmental conditions for culture of such cells.

Karyologic characterization of all cells maintained in the repository continues and specific collaborative programs requiring the use of karyologic data are in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH: The contractor has an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators, among others. (Text Abridged.)

222. DEVELOPMENT OF A CELL BANK FOR BOWEL CANCER RESEARCH

Hay, R. J., American Type Culture Collection, Dept. of Cell Culture, *12301 Parklawn Dr., Rockville, Maryland*, 20852, United States of America

The objectives of this program are to develop and otherwise obtain, characterize, expand, bank and distribute cell lines of use for studies on cancer of the large bowel. Work with tissues of human and laboratory animal origins is involved.

A committee was organized, and met in July to discuss the program. This group now consists of four members; the principal investigator (Robert J. Hay), D. Chopra, Ph.D.; John S. MacDonald, M.D., and Marvin M. Romsdahl, M.D., Ph.D. The Committee will examine credentials of lines banked before their release to the general scientific community.

Twenty four cell lines from human colorectal tumors and one cell line (CMT93) from a chemically induced tumor of the mouse have been received. The human lines include LoVo; SW48, 403, 480, 620, 797, 802, 837, 948, 1116, 1222, 1417, 1463; COLO 201, 205, 320DM, 320 HSR, 321 HSR; WidR; HuCCL-14; ME(ACR(plus)-S), PF(ACR(plus)-As); DLD1 and HCt 15. Lines shown to be free of adventitious agents have been or are being expanded from token freezes to produce larger seed and distribution stocks of ampules. Progeny from these stocks have or are being characterized further with regard to karyology, isoenzymology, tumorigenicity, growth properties, antigenic markers, and ultrastructure as appropriate. Five such seed-distribution stocks are currently in the final stages of characterization.

Isolation and cell line development work has been conducted on normal tissues from 39 humans and one Rhesus monkey, and on 18 human colon, rectal or cecal tumors. Eighteen of the tissues studied yielded cell lines which could be propagated sufficiently to warrant token freezing in liquid nitrogen for further separation and characterization efforts.

223. CORE FACILITY - TISSUE CULTURE AND CELL BANK

Acton, R. T., University of Alabama in Birmingham, School of Medicine, Dept. of Microbiology, *1919 7th Ave. S., Birmingham, Alabama*, 35233, United States of America

This is part of a broader project. A summary of this subproject is not available.

224. CHEMOTHERAPY TISSUE CULTURE FACILITY

Cole, J. W., Yale University, School of Medicine, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

This proposal supports a facility designed for the preparation of tissue culture media. The passage of uninfected culture lines of various sorts and maintenance of viable frozen lines of a variety of normal cells and the availability of media, maintenance of frozen cells and large-scale growth of cells for individual experiments are provided as services to members of the Cancer Center.

225. TISSUE CULTURE MEDIUM CORE

Sato, G., University of California, San Diego Campus, School of Medicine, Dept. of Medicine, *P.O. Box 109, La Jolla, California*, 92093, United States of America

This is part of a broader project. A summary of this subproject is not available.

226. PREPARATION OF FRESH AND CULTURED MAMMALIAN CELLS

Hay, R. J., American Type Culture Collection, *12301 Parklawn Dr., Rockville, Maryland*, 20852, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

227. WORKSHOP ON ANIMAL CELL CULTURE IN SERUM-FREE MEDIUM

Watson, J. D., Cold Spring Harbor Lab. of Quantitative Biology, *P.O. Box 100, Cold Spring Harbor, New York*, 11724, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

INVESTIGATOR INDEX

Aaronson, S.A. 143
Abraham, I. 186
Acton, R.T. 223
Adams, A.T. 112
Aden, D.P. 27
Aglietta, M. 65
Alsaker, R.D. 53
Amare, M. 47
Andrews, P. 27
Arimura, G.K. 100
Arnason, B.G. 84
Asato, N. 121
Ascoli, M. 108
Athwal, R.S. 2
Auersperg, N. 112
Austin, S.A. 173
Badaracco, G. 125
Baker, R.S. 120
Baldi, M.I. 159
Balinska, M. 182
Ballmer, K. 214
Barranco, S.C. 130
Barrett, J.C. 123
Baum, S.G. 98
Benassi, M. 125
Bertolini, L. 30
Bertram, J.S. 198
Biedler, J.L. 78
Bigner, D.D. 82
Biswas, D.K. 179
Blackstein, M.E. 89
Bluthmann, H. 23
Bode, U. 48
Boesen, E.A. 111
Bolund, L.A. 217
Bootsma, D. 152
Borenfreund, E. 199
Bosman, C. 30
Boss, M. 94
Botchan, P. 4
Bouck, N.P. 148
Bowden, G.T. 158
Braginski, J.E. 109
Brahe, C. 30
Bramwell, M.E. 18
Brattain, M.G. 103
Brinkley, B.R. 71
Buick, R.N. 115
Burger, M.M. 214
Burki, K. 23
Businaro, R. 30
Butler, R.H. 30
Buultjens, T.E. 185
Cabral, F. 186
Calabresi, F. 125
Calvelli, T.A. 122
Carbonell, A.W. 34
Carritt, B. 176
Cavelier, C. 195
Cha, S. 188
Chamberlain, J. 206
Chasin, L.A. 9
Chen, H.W. 140
Chu, E.H. 1
Chung, A.E. 109
Claisse, P.J. 200
Clarke, S.M. 106
Clayton, B. 187
Clemens, M.J. 173
Cohen, C. 17
Colbert, D. 48
Cole, J.W. 224
Colman, N. 190
Cooke, B.A. 213
Coosen, R. 87
Corringham, R.E. 111
Corsi, A. 125
Costa, A. 129
Cowell, J.K. 22
Craig, I.W. 154
Craighead, J.E. 96
Creighton, A.M. 32
Croce, C.M. 4, 141
Croft, W. 116
Curran, T. 51
Curren, R. 139
Dahl, O. 83
Daidone, M.G. 129
Danes, B.S. 99
Darlington, G.J. 5
Das, K.C. 190
Davidson, L.A. 120
Dawson, G.S. 6
de Jong, P.W. 87
de Koning, J. 67
Dei, T. 29
Deisseroth, A. 48
DeMars, R. 116
DeMars, R.I. 153
Dermody, W.C. 177
Devine, P.C. 106
Dexter, D.L. 102
Donelli, A. 55
Dougall, D.K. 211
Drago, J. 118
Duboule, D. 20
Duffy, P.E. 80
Durbin, H. 12, 34
Duval, D.P. 68
Dysart, J.M. 209
Ebisuzaki, K. 157
Edgar, D.H. 32
Edwards, J.G. 209
Elias, J. 90
Elias, Z. 195
Elkind, M.M. 136
Epstein, A.L. 14
Epstein, J. 43
Fay, J.W. 49
Felix, J.S. 8
Fernandez Pol, J.A. 210
Ferrari, S. 55
Field, J.K. 36, 86
Fleckman, P.H. 189
Fogh, J.E. 219
Fontana, J. 48
Forman, D. 193
Fox, M. 191
Franchini, G. 55
Francis, G.E. 46
Franks, L.M. 12
Frearson, P.M. 156
Freeman, J.G. 172
Fridland, A. 165
Gail, M. 138
Galivan, J.H. 182
Gallo, E. 65
Gaminio, G.D. 60
Gandini Attardi, D. 159
Gardner, M.B. 138
Gavilondo Cowley, J. 128
Gavosto, F. 65
Gelboin, H.V. 188
Gerschenson, L.E. 113
Geurts van Kessel, A.H. 152
Ghelardini, P. 30
Gielen, J. 201
Gilbert, A. 77
Gingrich, R. 18
Ginsberg, B.H. 88
Glaser, R. 124
Glode, L.M. 204
Goldman, R.D. 119, 145
Gonzalez, C.R. 60
Gottesman, M.M. 186
Graf, L.H. 122
Granner, D.K. 88
Graves, J.M. 6, 21
Greco, C. 125
Green, C.D. 36, 86
Green, H. 192
Gregson, N.A. 79
Grippo, J. 180
Grisham, J.W. 104
Gullino, P.M. 178
Gutierrez, R.M. 60
Hagemeijer, A.H. 152
Hales, A.C. 66, 216
Hallowes, R.C. 90
Hamburger, A.W. 50
Harmon, R.C. 31
Harris, H. 18
Hart, R.W. 92
Hartmann, D. 61
Hastings, R.J. 34, 149
Haugen, A. 83
Hauri, H. 202, 205
Hay, R.J. 222, 226
Henderson, E.E. 56
Hendrick, D. 48
Henry, S. 168
Herbert, V. 190
Herzenberg, L.A. 162
Heyes, J. 66
Higgins, P.J. 199
Hince, T.A. 200
Hochstadt, J. 181
Hodge, L.D. 168
Hohmann, P.G. 175
Holley, R.W. 208
Homo, F.G. 68
Hoogstraten, B. 47
Hooper, M.L. 185
House, W. 66, 216
Hudson, L. 64
Hudson, R.W. 105
Ilgren, E.B. 40
Illmensee, K. 20, 23

Ilmensee, R. 20
Isselbacher, K.J. 202, 205
Itoh, N. 72, 97
Jha, K.K. 144
Johnson, A.H. 106
Jonasson, J.A. 16
Jones, K.W. 13
Jones, W. 90
Jordan, P.W. 212
Kabat, E.A. 117
Kalckar, H.M. 183
Kamarck, M.E. 26, 57
Kan, Y. 48
Kandutsch, A.A. 140
Kaufman, D.G. 104
Kawamura, K. 69
Keech, C.T. 79
Kennett, R.H. 39
Kinkade, J.M. 63
Kinross, J. 13
Kirby, P. 156
Klein, G. 161
Klinger, H.P. 24, 38
Knaap, A.G. 146
Knazek, R.A. 178
Knowles, B.B. 27
Korn, D. 167
Korsgaard, R. 150
Kuppers, R.C. 54
Ladaga, L.E. 106
Laerum, O.D. 83
Lage Davila, A. 128
Lambert, D.T. 121
Latt, S.A. 151
Laughlin, J.S. 131
Leach, K.L. 180
LeCam, A. 186
Ledbetter, J.A. 162
Legg, A. 163
Lemons, R. 17
Lewis, C.W. 82
Lewis, M. 109
Leytus, S. 137
Libby, P.R. 198
Lieberman, M.W. 197
Lim, R. 84
Ling, V. 110
Liotta, L.A. 178
Littlefield, J.W. 8
Liu, H.Y. 137
Liu, J. 101
Ludwig, C. 61
Luftig, R.B. 172
MacDonald, C.M. 185
MacIndoe, J.H. 88
Maddock, C. 35
Madhav, R. 109
Maehle, B. 83
MageeBrown, R. 213
Maitland, N. 13
Malvehy, R.M. 162
Mandahl, N. 150
Mangel, W.F. 137
Maniatis, T. 48
Marshall Clarke, S. 74
Maruyama, K. 69
Matera, I. 65
Matsukage, A. 166
Mauro, F. 125
May, R.J. 202, 205
McBurney, M.W. 41
McCormick, P.J. 198
McCormick, W. 177
McKeehan, W.L. 107
MeherHomji, K. 120
Melhato, I. 137
Merchant, D.J. 106
Meuth, M.L. 134, 155
Midander, J. 132
Millerd, R.E. 64
Miller, H. 188
Miller, O.J. 19, 117
Miller, R.G. 73
Moldeus, P. 164
Mork, S. 83
Moses, H.L. 147
Mossman, B.T. 96
Munro, E. 154
Nagao, K. 143
Nakajima, Y. 69
Narni, F. 55
Nash, W. 138
Neely, J.E. 76
Nelson Rees, W. 138, 221
Neuwald, P.D. 169
Newman, R.A. 94
Nicolas, J. 186
Norback, D. 116
Nordenskjold, M. 164
Nose, K. 72, 97
Novarino, A. 65
O'Brien, S.J. 138
O'Farrell, M.K. 174
O'Neill, C.H. 212
Obrecht, J.P. 61
Oikarinen, A.I. 170
Okamoto, H. 72, 97
Olsson, I. 44
Otsuka, A. 159
Ozer, H.L. 144
Pagliardi, G.L. 65
Pain, C. 93
Park, C.H. 47
Park, S.S. 188
Parkhouse, R.M. 74
Parkinson, E.K. 196
Parks, D.R. 162
Peachey, L.A. 90
Perez, R. 128
Perry, P.E. 194
Peterson, W.D. 220
Petrovic, A. 3
Pettican, P. 163
Petzoldt, U. 20, 23
Phillips, S.G. 117
Pledger, W.J. 207
Plotkin, D.J. 142
Pollard, J.W. 206
Poortman, J. 87
Prasad, R. 181
Pratt, W.B. 180
Puck, T. 14
Quaroni, A. 202, 205
Quesenberry, P.J. 52
Rajan, T.V. 75
Ralph, P. 70
Rapp, F. 118
Ratcliffe, L. 66
Reed, S.I. 160
Reid, L.C. 95
Revesz, L. 132
Revoltella, R.P. 30
Reznikoff, C. 153
Reznikoff, C.A. 116
Rice, R.H. 114
Riddle, P.N. 90, 212
Ritter, M. 93
Robinson, R. 188
Robinson, W.A. 45
Rodgers, A. 18
Roscoe, J.P. 200
Rose, S. 215
Rosen, O.M. 171
Roth, C. 186
Royston, I. 59
Rozengurt, E. 163
Ruddle, N.H. 58
Rudland, P.S. 93, 94
Russell, T.R. 42
Rutherford, T. 48
Ryan, R.J. 147
Ryhanen, L.J. 170
Sacchi, A. 125
Samano, G.G. 60
Sanavio, F. 65
Sanfilippo, O. 129
Santhanan, S. 116
Sato, G. 225
Schall, D.G. 147
Schultz, D.R. 100
Schwarz, F. 87
Schwarz, G. 133
Searls, D.B. 28
Sery, T.W. 127
Shapiro, W.R. 81
Sherman, B.M. 88
Sherr, C.J. 17
Sherratt, D.J. 176, 185
Shows, T.B. 15
Silagi, S. 122
Silvestrini, R. 129
Siminovitch, L. 7
Simon, M. 192
Simons, J.W. 146
Singh, T. 186
Siniscalco, M. 3
Siskin, E.E. 123
Slack, J.M. 193
Solter, D. 27
Southgate, J. 22
Stammatoyannopoulos, G. 48
Stampfer, M.R. 91
Stanners, C.P. 206
Starkey, J. 11
Starkey, J.R. 203
Stroller, D. 33
SubakSharpe, J.H. 176, 185
Sueoka, N. 85
Surti, U. 138
Swart, K. 152
Swift, M.R. 184
Tachibana, T. 29
Takahashi, T. 166
Talley, F. 123
Tan, E.M. 170
Tanaka, N. 69
Teich, N. 51
Thijssen, J.H. 87
Thirion, J.P. 10
Thomson, E.J. 194
Thomson, R. 156
Tilly, R.J. 193
Tischfield, J. 48
Tom, B.H. 101
Torelli, G. 55
Torelli, U.L. 55
Torre, A. 30
Toyston, I. 25
Tsuzuki, J. 172
Ueno, T. 69
Ultto, J.J. 170
van 't Veer Korthof, E. 67
van de Poll, K.W. 135
van Rijn, J. 135
van Weel Sipman, M.H. 67
van Wijk, R. 135
van Zeeland, A.A. 146
Varga, J.M. 121
Vedros, N. 221
Vigenti, E. 30
Villanueva, T.I. 60
Vlahakis, G. 186
Vogler, W.R. 63
Wang, T.S. 167

Wang, Y.J. 84
Watson, J.D. 227
Waye, M. 206
Weatherall, D. 48
Weatherbee, J.A. 172
Weihing, R.R. 172
Whitten, G.H. 211
Wiedmann, T.W. 214
Wieman, M. 218
Wigley, C.B. 35, 126
Winton, E.F. 63
Wouters, M. 18
Wright, D. 48
Wu, M. 100
Yamaguchi, M. 166
Yount, W.J. 37
Yung, Y.P. 70
Yunis, A.A. 100
Zackroff, R.V. 119
Zarling, J.M. 62
Zuniga, M.C. 58
Zupi, G. 125

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D. If you are submitting a clinical protocol, please send a copy of the complete protocol. The NCI has an established format which will be used to develop a standard protocol summary. A copy of the prepared summary will be sent to you for review prior to its entry into the data base.

E. Return to address shown on the front of this form.

F. IMPORTANT. If you prefer to have your copy of the SPECIAL LISTING mailed to your home address, or if your co-investigators have different mailing addresses, please list each name and address on a separate sheet of paper.

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